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(54) Title: METHODS AND REAGENTS FOR TYPING HLA CLASS I GENES

(57) Abstract

Consensus sequences of introns (1, 2 and 3) from the majority of HLA-A, -B and -C allotypes are identified and used to develop primers located within introns (1 and 3) of these genes. The primers are suitable for locus-specific amplification of the entirety of exons (2 and 3). These primers are also suitable for use as sequencing primers to determine the HLA alleles in sequence-based HLA typing. The primers can be used for amplification of portions of introns 1 or 3 and evaluating the amplified products to determine the allelic type of the HLA-A, HLA-B or HLA-C genes. Preferably, one of the primers has a sequence which provides locus-specific amplification.

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METHODS AND REAGENTS FOR TYPING HLA CLASS I GENES

DESCRIPTION

This application relates to methods and reagents for typing HLA alleles of Class I genes.

The HLA Class I genes are a component of the human major histocompatibility complex (MHC). The Class I genes consist of the three classical genes encoding the major transplantation antigens HLA-A, HLA-B and HLA-C and seven non-classical class I genes, HLA-E, HLA-F, HLA-G, HLA-H, HLA-J, HLA-K and HLA-L.

The classical HLA Class I genes encode polymorphic cell surface proteins expressed on most nucleated cells. The natural function of these proteins is to bind and present diverse sets of peptide fragments from intracellularly processed antigens to the T cell antigen receptors (TCRs). Thus, the peptide-binding capability of the MHC molecule facilitates immune recognition of intracellular pathogens and altered self proteins. Therefore, by increasing the peptide repertoire for TCRs, the polymorphism of MHC molecules plays a critical role in the immune response potential of a host. On the other hand, MHC polymorphism exerts an immunological burden on the host transplanted with allogeneic tissues. As a result, mismatches in HLA class I molecules are one of the main causes of allograft rejection and graft versus host disease, and the level of HLA matching between tissue donor and recipient is a major factor in the success of allogeneic tissue and marrow transplants. It is therefore a matter of considerable medical significance to be able to determine the "type" of the HLA Class I genes of candidate organ donors and recipients.

HLA class I histocompatibility antigens for patient-donor matching are conventionally determined by serological typing. Biochemical and molecular techniques have revealed that HLA class I polymorphism is far greater than previously recognized by conventional methods. To date, over 59 HLA-A, 127 HLA-B, and 36 HLA-C different allelic sequences have been identified. Bodmer et al., "Nomenclature for factors of the HLA system," *Tissue Antigens* 46: 1-18 (1995). This high level of allelic diversity complicates the typing of the HLA class I genes.

Another complicating factor is the large number of homologous genes and alleles. Each of the HLA Class I genes is composed of eight exons and seven introns as shown in Fig. 1, and the sequences of these exons and introns are highly conserved across the HLA

Class I genes. Allelic variations mostly occur in exons 2 and 3 which are flanked by noncoding introns 1, 2, and 3. These two exons encode the functional domains of the molecules.

Taken together, these two complications make HLA Class I typing at the nucleic acid level a formidable task. Allelic diversity within any one gene means that a great many probes need to be developed if hybridization-based tests are used in the typing. Further, the general applicability of DNA typing methods to HLA Class I genes depends on the design of primers which provide effective locus-specific amplification of exons 2 and/or 3 of one HLA Class I gene.

One method for performing HLA Class-I typing is disclosed in US Patent No. 5,424,184 which is incorporated herein by reference. This patent utilizes primers which are located within exons 2 and 3 of the HLA Class-I genes to achieve what is described as group-specific amplification of a portion of the HLA-A, HLA-B and HLA-C genes. This approach is not ideal, however, since the primers hybridize with portions of the coding strand, and thus may mask significant allelic variations. In addition, this method requires a grouping of alleles by means of another method in order to select group-specific primers for amplification.

In assessing the known exon 2 and 3 sequences found in the HLA class I sequence database (Arnett & Parham, *Tissue Antigens* 46: 217-257 (1995)), there is only one possible HLA-A locus-specific primer site located in exon 2. (Oh et al., *Tissue Antigens* 41: 135-142 (1993)) Using a primer for this site, HLA-A locus-specific amplification produced a PCR product of 671bp, containing a portion of exon 2, intron 2, and exon 3. This amplified DNA fragment does not contain the first variable region of HLA-A the molecule. In addition, the primers are not entirely specific and lead to amplification of some HLA-H alleles. Thus, locus-specific amplification using this primer does not provide a highly effective method for typing HLA-A genes.

Similar evaluations of the known exon sequences (Arnett and Parham, *supra*) showed that there are no suitable primer sites for the HLA-B genes. For HLA-C alleles, two separate sets of primers are needed to amplify both exon 2 and 3. (Levine and Yang, *Tissue Antigens* 44: 174-183 (1994). Universal primers designed for exons 2 and 3 also amplified

the non-classical genes. Exon 4 contains locus-specific sequences but it is separated by ≈590bp from exon 3, making exon 4 an impractical PCR primer site.

Thus, there remains a real need for locus-specific primers for the HLA class I genes to provide amplified materials for use in class I typing by PCR-DNA methods (e.g. Sequencing-, SSO-, and SSP-based). It is an object of the present invention to provide methods and reagents effective to provide locus-specific amplification of the HLA Class I genes.

It is a further object of the present invention to provide locus-specific primers amplification primers which hybridize with the introns flanking exons 2 and 3 of the major transplantation antigens.

It is still a further object of the present invention to use these primers to achieve locus-specific amplification which is an essential step in developing a DNA-based HLA class I typing methodology.

SUMMARY OF THE INVENTION

We have determined the sequences of introns 1, 2 and 3 from the majority of HLA-A, -B and -C allotypes and from alleles of HLA-E, -F, -G, -H, -J, -K, and -L. From these intron sequences, we have now developed primers located within introns 1 and 3 of the HLA-A, HLA-B and HLA-C genes. These primers are suitable for locus-specific amplification of the entirety of exons 2 and 3, i.e., the portion of these of genes most suitable for use in typing of HLA-A, HLA-B and HLA-C. These primers are also suitable for use as sequencing primers to determine the HLA alleles in sequence-based HLA typing. Thus, in accordance with the invention, there is provided a method for testing a sample to determine the HLA-A, -B or -C type of the sample comprising the steps of

(a) treating the tissue sample to obtain nucleic acid polymers suitable for amplification;

(b) combining the nucleic acid polymers with a first primer which is complementary to a portion of intron 1 of the HLA gene, and a second primer which is complementary to a portion of intron 3 of the HLA gene under conditions suitable for amplification to obtain an amplified product; and

(c) evaluating the amplified product to determine the allelic type of the HLA-A, HLA-B or HLA-C genes. This evaluation step can make use of any of the known methods

for nucleic acid-based typing of HLA genes, including direct sequencing, sequence-specific oligotyping (SSO) or sequence-specific primer amplification (SSP) of the amplified products. Preferably, at least one of the amplification primers has a sequence which provides locus-specific amplification.

In addition, we have identified primers which provide locus-specific amplification for HLA-E, HLA-F, HLA-G, HLA-H, HLA-J, HLA-K and HLA-L. Thus, there is also provided a method for testing a tissue sample to determine the HLA-E, HLA-F, HLA-G, HLA-H, HLA-J, HLA-K and/or HLA-L type of the sample comprising the steps of

(a) treating the tissue sample to obtain nucleic acid polymers suitable for amplification;

(b) combining the nucleic acid polymers with a first primer which is complementary to a portion of exon 2 of the human major histocompatibility complex, and a second primer which is complementary to a portion of exon 3 of the human major histocompatibility complex under conditions suitable for to obtain an amplified product; and

(c) evaluating the amplified product to determine the allelic type of the HLA-E, HLA-F, HLA-G, HLA-H, HLA-J, HLA-K and HLA-L genes. This evaluation step can make use of sequence-specific oligotyping, PCR-SSOP-based typing or can involve direct sequencing the amplified products.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the organization of the eight exons and seven introns of an HLA Class I gene;

Fig. 2 shows a consensus sequence for intron 1 of the classical HLA Class I genes with suggested primer locations;

Fig. 3 shows a consensus sequence for intron 2 of the classical HLA Class I genes with suggested primer locations;

Fig. 4 shows a consensus sequence for intron 3 of the classical HLA Class I genes with suggested primer locations;

Figs. 5A-5H shows individual aligned sequences determined for each intron;

Fig. 6 shows the sequences of exon 2 of the non-classical HLA Class I genes;

Fig. 7 shows the sequences of exon 3 of the non-classical HLA Class I genes; and

Fig. 8 shows the name and allelic type of 106 cell lines tested using the amplification primers of the invention.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to methods for typing tissue samples to determine the HLA Class I type of the sample. Thus, a first embodiment of the invention is a method for testing a tissue sample to determine the HLA-A, HLA-B or HLA-C type of the sample comprising the steps of

(a) treating the tissue sample to obtain nucleic acid polymers suitable for amplification;

(b) combining the nucleic acid polymers with a first primer which hybridizes with a portion of intron 1 or intron 3 of the HLA-gene being tested, and a second primer which hybridizes with a different portion of the HLA-gene being tested under conditions suitable for nucleic acid amplification to obtain an amplified product; and

(c) evaluating the amplified product to determine the allelic type of the HLA-A, HLA-B or HLA-C genes. Preferably, at least the first amplification primer is one which specifically hybridizes to only one type of HLA Class I gene, so that locus specific amplification is achieved.

Figs. 2, 3 and 4 shows combined sequences (Seq. ID Nos.: 1-9) for introns 1, 2 and 3 respectively, together with suitable locations for binding amplification primers. These sequences are consensus sequences derived from the individual aligned sequences determined for each intron as shown in Figs. 5A-5H. In these sequences, bases which are the same for the locus across the various strains tested are indicated as a single base (A, C, G or T), while bases which were variable in the strains tested are indicated by a code for alternative bases. In general, it will be advantageous to select primers to avoid the variable bases, although in some of the primers discussed below, intra-locus variation is taken into account.

The method of the invention can be performed on whole blood, tumor cells, sperm, hair follicles or any other nucleated tissue sample.

Once the sample is obtained, the next step is to treat the tissue sample to obtain nucleic acids for amplification. Genomic DNA preparation suitable for amplification can be obtained by proteinase K digestion, as previously described in Levine et al., *Tissue Antigens*

44: 174-183 (1994). Briefly, this method involves removal of red cells in the case of blood samples after lysing by a hypotonic solution and then the remaining white cells are treated with Proteinase K in a detergent-containing solution to release DNA from nuclei and digest proteins in the cell lysate. After inactivation of the proteinase K, the remaining DNA in the solution is used as an amplification template. Other methods for preparing genomic DNA which may also be used in accordance with the invention include salting-out extraction procedures (Miller S, Dykes D, and Polesky H., "A simple salting out procedure for extracting DNA from human nucleated cells" Nucleic Acids Res. 16: 1215, 1988) and the standard phenol-chloroform DNA extraction procedure (Current Protocols in Molecular Biology, Series ed. K. Jansson, Wiley Interscience).

Once the sample has been treated, it is combined with two amplification primers and amplified, for example using Polymerase Chain Reaction (PCR) amplification. The basic process of PCR amplification is known, for example from U.S. Patents Nos. 4,683,202 and 4,683,195, which are incorporated herein by reference. In PCR amplification, two amplification primers are used, each of which hybridizes to a different one of the two strands of a DNA duplex. Multiple cycles of primer extension, and denaturation are used to produce additional copies of DNA extending from the position of one primer to the position of the other. In this way, the number of copies of the genetic material positioned between the two primer binding sites is increased.

In the present invention, amplification of exons 2 and 3 is preferably performed using at least one locus-specific primer which specifically hybridizes to a portion of intron 1 or intron 3. As used in the specification and claims hereof, the primers which "specifically hybridize" to the introns are primers which permit locus-specific amplification by having a sequence which is exactly complementary to the expected sequence of a portion of the intron so that binding and amplification can occur, but which is not complementary to a region on any of the other HLA Class I genes. It will be understood that locus-specific primers within the scope of this invention need not be complementary to a totally unique sequence within the human genome, provided that both members of the primer pair used in amplification do not bind to the same gene outside the gene of interest.

The second amplification primer is preferably one which hybridizes with the other flanking intron (i.e., intron 3 when the first primer hybridizes to intron 1 and vice versa),

since this will result in the simultaneous amplification of both exons 2 and 3. It will be appreciated, however, that exons 2 and 3 could be amplified individually by selecting a second amplification primer for exon 2 and a first primer for exon 3 which hybridize with intron 2 (Seq. ID Nos.: 2, 5 and 8), and such amplifications are within the scope of the invention.

Amplification primers useful in the present invention are generally from 10 to 40 bases in length, more preferably from 21 to 35 bases in length. Within this size range, we have identified suitable locus-specific, group specific and allele-specific primers for each of the classical HLA Class I genes.

For locus-specific amplification of the HLA-A gene, suitable locus-specific primers have the sequence

GGCCTCTGYG GGGAGAAGCA A SEQ ID NO.: 10

or

GAAACSGCCT CTGYGGGGAG AAGCC SEQ ID NO.: 11

Degenerate bases can be introduced in the primer sequences where alternative bases occur among alleles. These primers are complementary to the region of the non-coding strand spanning nucleotides 26-46 and 21-45, respectively of the intron 1 sequence shown in Fig. 2 (Seq. ID No.: 1). It will be appreciated that this primer could be made longer by adding additional complementary bases to the 5'-end. The primer might also be made somewhat shorter, for example spanning nucleotides 26-44, since nucleotides 23, 24 and 25 are identical across the various HLA-locuses in sequences of which the inventors are aware. In addition to primers binding to the non-coding strand, it will be appreciated that complementary primers which bind to the corresponding portions of the coding strand could be used with a compatible second primer. The use of longer or shorter locus-specific primers, and of complementary locus-specific primers are within the scope of the present invention.

Locations of these and additional primers within each of introns 1, 2, and 3 are shown in Figs. 2, 3 and 4.

An amplification primer which binds to the non-coding strand of the HLA-A gene is used in combination with a second amplification primer which binds to the coding strand to achieve locus specific amplification. Preferably, both primers will be locus-specific in their

hybridization to the HLA gene, although to achieve locus specific amplification only one of the two primers used to amplify DNA from the sample is required to be a locus-specific primer. Examples of locus-specific amplification primers which bind to the coding strand of the HLA-A gene include

CGGGAGATCT AYAGGCGATC AGG SEQ ID No.: 12,

TGTTGGTCCC AATTGTCTCC CCTC SEQ ID No.: 13, and

AGGATTCCTC TCCCTCAGGA CCAG SEQ ID No.: 14.

These primers bind to the region of the coding strand of intron 3 of the HLA-A gene (SEQ ID No.: 3) spanning nucleotides 25-47, 65-88 and 108-131, respectively, as shown in Fig. 4. As in the case of the first amplification primer, amplification primers which are a made a few bases longer by virtue of adding additional complementary bases, amplification primers which are a few bases shorter, and complementary amplification primers may be used in the method of the present invention. Other potential sites for HLA-A locus specific primers are highlighted in Fig. 4.

The amplification primers and the genomic DNA are combined in an amplification mixture, for example containing 10 to 100 ng of genomic DNA in a 100 µl volume containing 0.2 mM dNTPs, the two primers at a concentration of 0.2 µM each, 2.5 units of Taq polymerase, 50 mM Tris-HCl (pH 8.8), 50 µM EDTA, 1.5 mM $MgCl_2$, 0.01% (w/v) gelatin, 10 mM β-mercaptoethanol and 10% (w/v) DMSO. The mixture is denatured at a temperature of 96°C of 5 minutes. Multiple cycles, for example thirty cycles, of amplification are then performed. For HLA-A, -B and -C a suitable cycle program is

denaturation	94°C	22 seconds
annealing	65°C	50 seconds
extensions	72°C	30 seconds.

Different cycling conditions may be used to obtain good PCR yields from longer or shorter primers.

While PCR amplification is the preferred approach to amplification of the treated sample, other techniques which use oligonucleotide primers to define a region of DNA to be amplified can be used as well. Such techniques include ligase chain reaction amplification (Wiedmann et al., PCR Primer, Laboratory Manual, Cold Spring Harbor (1991)).

The amplification procedure results in the production of an amplified product, in which the region of the HLA-A gene between the two primers is significantly increased in concentration relative to other genetic material in the treated sample. The amplified product is then evaluated to determine the allelic type of the HLA gene. This evaluation step can utilize any procedure which results in identification of allelic type.

For example, the amplification product can be evaluated by hybridization with locus-specific, group- specific or allele-specific oligonucleotide probes. Probes of this type which bind to the HLA-A gene are known in the art, for example from Oh et al., *Tissue Antigens* 41: 135-142 (1993) and Bugawan et al., *Tissue Antigens* 44: 137-147 (1994).

Oligonucleotide probes can be used in any of a number of test formats. For example, a dot blot analysis can be performed as described in Examples 1-3 below. Briefly, in this analysis the amplified product is affixed to a solid support in an array of dots. Labeled probes of different types are then applied to the dots. After washing to remove unhybridized probes, each dot is evaluated for the presence of hybridized (bound) probe using the label.

Other hybridization test formats may also be used. For example, the amplification primers used may be labeled with a detectable label, e.g., a radiolabel, a colored or chromogenic label, or a fluorescent or fluorogenic label; or an immobilization moiety such a biotin. The probes are then labeled with a complementary type of label, i.e., immobilizing when the amplification primers have a detectable label, and a detectable label when the amplification primers are immobilizable. The probes and the amplification products are combined under hybridizing conditions before or after immobilization of the immobilizable component of the reaction on a solid support, and the capture of the labeled component onto the solid support is monitored. Suitable solid supports include chromatographic columns and magnetic beads. Specific examples of suitable probes are listed in Table 1.

The amplification product may also be evaluated using direct sequencing as described in Santamaria et al., *Hum. Immunology* 37: 39-50 (1993). The amplified product can be sequenced using the well-known dideoxy chain termination method. Briefly, in this method a sequencing primer complementary to one strand of the amplified product is combined with the amplified product, a template-dependent polymerase enzyme, a mixture of the four standard nucleotide bases (A, G, T, and C) and one type of dideoxy nucleotide base. The

TABLE 1

SEQ ID No.	Probe *=noncoding	Sequence	First Codon Pos.	Specificity
62	131R	CGCTCTTGGA CCGCG	131	A, L
63	HBB034	GTTCGTGAGG TTCGACAGC	32	B
64	HYB035	CGCCGTGGGT GGAGCAGGA	49	B*5401, C, G, L
65	EE2-210	GCACAGACAC GGAACACC	71	E
66	FE2-200*	GTCTGTCCGT TGGCCTTG	67	F
67	GE2-183	GAGGAGACAC GGAACACC	62	G, L
68	HE3-479*	TCCACGAACT CGCCCTCC	158	H
69	JE3-274*	TTCCCTGGAG GATGTGAT	92	J, K
70	HLB032	CAGCGACTCC GTGAGTCCG	37	L
71	142IK	CAGATCACCA AGCGC	141	A1, A3, A11, A24, A36, H
72	114EH	TATGAACAGC ACGCC	113	A30, H
73	HXC008	CTGCGGATCG CGCTCCGCT	78	A23, A24, A25, A32, B*2702, B38, B49, B51, B52, B53, B17, H
74	HBB055	CCGCGAGTCC GAGGATGGC	40	B15, B46, B57, E
75	HBC009	CTGCGGACCC TGCTCCGCT	78	B27, B37, B47, J, K, L
76	HYE024	GGACCTGCGC TCCTGGACC	128	B7, B8, B*2707, B40, B41, B42, B*4801, all Cw's except Cw3, Cw4, and Cw14, E, F, G, H, J, K, L
77	HBD080	CGGGTACCAC CAGGACGCC	111	B27, B47
78	HBD083	CGGGTATGAC CAGGACGCC	111	B44
79	HBD086	CGGGTATAAC CAGTTAGCC	111	B45, B49, B50
80	HBF094	GACAAGCTGG AGCGCGCTG	177	B7, B*4001, B*4802
81	HBC065	GAAGTACAAG CGCCAGGCA	65	B46
82	HBC066	GAACATGAAG GCCTCCGCG	65	B57, B58
83	156R	GCGGAGCAGC GGAGAGCC	153	B7, B*3508, B*5702, Cw1, Cw*0401, Cw*0802, Cw14

bases are added to the end of the amplification primer to form a new oligonucleotide complementary to the amplification product. When a dideoxy base is added, however, no additional bases can be added. This results in the formations of a family of oligonucleotides whose lengths reflect the positions of the nucleotide base provided in dideoxy form within the complementary oligonucleotide. By evaluating the fragments formed in four reactions mixtures, one for each type of dideoxy nucleotide base, by gel electrophoresis, the sequence of the complementary strand can be deduced.

Basic procedures for performing nucleic acid sequencing in this manner are well known in the art, and commercial instruments are available for this purpose. Thus, sequencing is a routine procedure provided that amplified DNA and suitable primers are available. In this case, the same primers used to amplify the DNA can be used as sequencing primers.

Nested intron primers can also be used as sequencing primers. These primers are complementary to the sequences of the amplified products located in intron 1, intron 2 or intron 3 (SEQ ID Nos.: 1-9). It is particularly advantageous to have "universal" sequencing primers which could be used in the sequencing of any of the major transplantation antigen genes after locus-specific amplification, and such primers are an aspect of the present invention.

Examples of universal primers for sequencing the non-coding strand of the exon 2 which are complementary to the non-coding strand of intron 1 include:

GGGTCKGKYR GRTYTCAGC SEQ ID No.: 15

and

CGCSCMKGGA SGWGGGTC SEQ ID No.: 16.

These primers are complementary to the portion of intron 1 spanning nucleotides 95-113 and 82-99, respectively.

An example of a universal primer for sequencing the non-coding strand of the exon 2 which is complementary to the non-coding strand of the exon 2 is

TCYCACTCCA TGAGGTATTT C SEQ ID No.: 17.

This primer is complementary to the portion of exon 2 spanning nucleotides 3-23.

Examples of universal primers for sequencing of the coding strand of the exon 2 which are complementary to the coding strand of intron 2 include:

GGCYGGGGTC ACTCACCG SEQ ID No.: 18

and

GTCSTGACCT SCGCCCC SEQ ID No.: 19.

These primers are complementary to the portion of intron 2 spanning nucleotides -2 to 15 and 19-35, respectively.

Examples of universal primers for sequencing of the non-coding strand of the exon 3 which are complementary to the non-coding strand of the intron 2 include:

GCGGGRCGGG GCTCGGGGG SEQ ID No.: 20

and

ATYCCCSCRG KTTGGTC SEQ ID No.: 21.

These primers are complementary to the portion of intron 2 spanning nucleotides 214-236 and 194-210, respectively.

An example of a universal primer for sequencing of the coding strand of the exon 3 which is complementary to the coding strand of the intron 3 is

CCCYRYKGCC CCTGGTAC SEQ ID No.: 22.

This primer is complementary to the portion of intron 3 spanning nucleotides 1 to 18.

Other potential sequencing primer sites are highlighted in Figs. 2, 3 and 4. In addition, the primers disclosed in US Patent No. 5,424,184 for sequencing of the HLA-A locus may also be used.

The amplified DNA products may also be evaluated by agarose gel electrophoresis for typing HLA alleles, for example using the techniques described in Browning et al., *Hum. Immunology* 39: 143 (1994); Krausa et al., *Lancet* 341: 121-122 (1993). Briefly, in this method each group of alleles or individual allele is amplified by a group-specific or an allele-specific primer pair exactly matched to that group or allele. By keeping the PCR conditions stringent, the primer pairs will not non-specifically amplify other related alleles. The amplification primers are designed with the specificity-dependent nucleotide(s) on the terminal 3'-prime end. Identification of the alleles is based on the absence or presence of amplified products observed after agarose gel electrophoresis.

The same procedures described above can be used in accordance with the invention to determine the type of HLA-B and HLA-C genes in a sample. For typing the HLA-B gene, an exemplary locus-specific first amplification primer has the sequence

GGGAGGAGCG AGGGGACCSC AG SEQ ID No.: 23

This amplification primer is complementary to the region of the non-coding strand spanning nucleotides 36-57 of the intron 1 sequence (Seq ID No.: 4) of the HLA-B gene shown in Fig. 2. Primers might also be derived from the di-allelic site spanning nucleotides 57-76, for example

CGGGGGCGCA GGACCCGG SEQ ID No.: 24

or

GGCGGGGGCG CAGGACCTGA SEQ ID No.: 25

which span nucleotides 59-76 and 57-76, respectively.

As in the case of the locus-specific primers for HLA-A, it will be appreciated that this primer could be made longer by adding additional complementary bases to either or both ends. The amplification primer might also be made somewhat shorter, for example spanning nucleotides 39-57 in the case of Seq ID No.: 23, since nucleotides 38, 39 are identical across the HLA-genes of which the inventors are aware, although this may result in the loss of some discrimination between HLA-B and HLA-C genes if nucleotide 37 (which is different in HLA-C genes from HLA-A and HLA-B) is not spanned by the probe. In addition, complementary amplification primers which bind to the corresponding portions of the coding strand could be used with a compatible second primer. The use of such longer or shorter primers and of complementary primers is within the scope of the present invention.

Exemplary locus-specific second amplification primers which can be used in typing the HLA-B gene using the method of the invention have the sequence:

GGAGGCCATC CCCGGCGACC T SEQ ID NO.: 26,

and

GGAGGCCATC CCCGGCGACC TAT SEQ ID No.: 27,

These primers bind to the region of the coding strand of intron 3 of the HLA-B gene (SEQ ID No.: 6) spanning nucleotides 38-58, 36-58, respectively, as shown in Fig. 4. As in the case of the first amplification primer, amplification primers which are a made a few bases longer by virtue of adding additional complementary bases, amplification primers which are a few bases shorter, particularly those which span nucleotides 40-50, and complementary amplification primers may be used in the method of the present invention.

The primers

CTCAGGAAAA CTCATSCCAT TCTCCATTC AAG SEQ ID No.: 28;

and

GGAGATGGGG AAGGCTCCCC ACT SEQ ID No.: 29

which bind to the region of the coding strand of intron 3 of the HLA-B gene (SEQ ID No.: 6) spanning nucleotides 106-137 and 12-34, respectively, can also be used for amplification of the HLA gene, although this primer also amplifies HLA-C.

Other locus-specific, group-specific or allele specific oligonucleotides which are complementary to the non-coding strand of introns 1 and 2 of the HLA-B gene and which can be used in the method of the invention are indicated in Figs. 2 and 3. Similarly, other locus-specific, group-specific or allele specific oligonucleotides which are complementary to the coding strand of introns 2 and 3 of the HLA-B gene and which can be used in the method of the invention are indicated in Figs. 3 and 4. Any of these oligonucleotides can be used in combination the locus-specific primers for locus-specific amplification, although the use of two-locus specific amplification primers is preferred. These oligonucleotides can also be used as sequencing primers for typing of the HLA-B gene, although the universal primers described above (SEQ ID Nos.: 15-22) are preferred.

Suitable probes for use in hybridization assays of the type of the amplified product made using these primers are Ragupathi et al., *Tissue Antigens* 46: 24-31 (1995), Fernandez-Vina et al., *Tissue Antigens* 45: 153-168 (1995); and in *Fleischhauer et al., Tissue Antigens* 46: 281-292 (1995) and are listed in Table 1.

For typing the HLA-C gene, the suitable locus-specific first amplification primers have the sequences:

AGCGAGGXGC CCGCCCGGCG A SEQ ID NO.: 30,

GAGGGAAACG GCCTCTGCGG A SEQ ID NO.: 31,

GAGGGGCCCG CCCGGCGA SEQ ID NO.: 32,

or

GACCCGGGGA GCCGCGCA SEQ ID NO.: 33.

These locus-specific amplification primers are complementary to the region of the non-coding strand spanning nucleotides 42 to 62 (SEQ ID No.: 30), 17-37 (SEQ ID No.: 31), 45-62 (SEQ ID No.: 32) and 71-88 (SEQ ID No.: 33)of intron 1 of the HLA-C gene sequence (SEQ ID No.: 7) as shown in Fig. 2. It will be appreciated that these

amplification primers could be made longer by adding additional complementary bases to either or both ends. The amplification primer might also be made somewhat shorter. The nucleotides complementary to nucleotides 34 and 37 for SEQ ID NO.: 31, 56 and 61 for SEQ ID NO.: 32, and 78 and 87 for SEQ ID NO.: 33 should be retained in the amplification primer, since these bases are distinct in the HLA-C intron from both HLA-A and HLA-B. In addition, complementary amplification primers which bind to the corresponding portions of the coding strand could be used with a compatible second amplification primer. The use of such longer or shorter amplification primers and of complementary amplification primers is within the scope of the present invention.

Suitable locus specific second amplification primers which are compatible to the coding strand include:

CGCTGATCCC ATTTTCCTCC CCTC SEQ ID NO.: 34,

GGAGATGGGG AAGGCTCCCC ACT SEQ ID NO.: 29,

CTCAGGAAAA CTCATSCCAT TCTCCATTCA AG SEQ ID NO.: 35,

ACCACAGCTG CTGCAGTGGT CAAAGTG SEQ ID NO.: 36,

GAGGAAAGGT CAGCAGCCTG ACCACA SEQ ID NO.: 37,

or

GACTCAGAAA AGCTGGAATC AAACCTT SEQ ID NO.: 38.

These locus-specific amplification primers bind to the region of the coding strand of intron 3 of the HLA-C gene (SEQ ID No.: 9) spanning nucleotides 65-88 (SEQ ID NO.: 34), 12-34 (SEQ ID No.: 29), 106-137 (SEQ ID No.: 35), 267-291 (SEQ ID NO.: 36), 283-304 (SEQ ID NO.: 37), 342-368 (SEQ ID NO.: 38), respectively, as shown in Fig. 4. As in the case of the first amplification primers, amplification primers which are a made a few bases longer by virtue of adding additional complementary bases, amplification primers which are a few bases shorter, and complementary amplification primers may be used in the method of the present invention. The primers SEQ ID Nos.: 28, and 29 39 are specific for both B and C loci, therefore this primers can be used for HLA-C amplification when it is paired with an HLA-C-locus-specific 5' primer such as SEQ ID NO.: 31, SEQ ID NO.: 32, or SEQ ID NO.: 33.

Suitable probes for use in hybridization assays of the type of the amplified product made using these amplification primers are described in Levine et al. *Tissue Antigens* 44: 174-183 (1994) and in Table 1.

A further aspect of the present invention is determination of the allelic type of the non-classical Class I genes, i.e., HLA-E, -F, -G, -H, -J, -K and -L. In this case, the primers which we have identified as providing the most unique locus specific amplification for this purpose are located with exon 2 and exon 3 of the respective HLA gene. These locus specific amplification primers are used in the same general manner as the amplification primers discussed above for HLA-A, with specific differences being noted below.

For typing the HLA-E gene, the first amplification primer has the sequence

CACTCCTTGA AGTATTTCCA CACT SEQ ID No.: 41

or

TGGAAACGGC CTCTACCGGG AGTAGAG SEQ ID No.: 42.

SEQ ID No.: 41 is complementary to the region of the non-coding strand spanning nucleotides 6-29 of exon 2 (SEQ ID No.: 39) of the HLA-E gene sequence shown in Fig. 6. SEQ ID No.: 42 is complementary to the region of the non-coding strand spanning nucleotides 19-45 of intron 1 of the HLA-E gene sequence (SEQ ID No.: 43).

It will be appreciated that these amplification primers could be made longer by adding additional complementary bases to either or both ends. The amplification primer might also be made somewhat shorter, for example spanning at least nucleotides 6-12 for SEQ ID No.: 41. Nucleotides complementary to nucleotides 24 and 29 for SEQ ID No.: 41 and 38-44 for SEQ ID NO.: 42 are also advantageously retained in a locus-specific primer since these bases are distinct in the HLA-E gene sequence from the rest of the genes. In addition, complementary amplification primers which bind to the corresponding portions of the coding strand could be used with a compatible second amplification primer. The use of such longer or shorter amplification primers and of complementary amplification primers is within the scope of the present invention.

Exemplary second amplification primers used in typing the HLA-E gene using the method of the invention have the sequence

TCTCCTTCCC CTTCTCCAGG TATT SEQ ID NO.: 44

or

CACAGTCCTA GCCCAAGAAG GAGATGGGAG AGTA SEQ ID No.: 45.

SEQ ID No.: 44 primer binds to the region of the coding strand of exon 3 of the HLA-E gene (SEQ ID No.: 40) spanning nucleotides 238-261 as shown in Fig. 7. SEQ ID No.: 45 primer binds to the region of the coding strand of intron 3 of the HLA-E gene (SEQ ID No.: 46) spanning nucleotides 19-53. As in the case of the first amplification primer, amplification primers which are a made a few bases longer by virtue of adding additional complementary bases, amplification primers which are a few bases shorter (retaining the 3'-end), and complementary amplification primers may be used in the method of the present invention.

Amplification of the HLA-E gene is performed using the same general methodology described for amplification of HLA-A, -B and -C genes. The cycle program in this case, however, is preferably

denaturation	94°C	22 seconds
annealing	62°C	50 seconds
extensions	72°C	30 seconds.

Suitable probes for use in hybridization assays of the type of the amplified product made using these amplification primers are listed in Table 1.

For typing the HLA-F gene, the first amplification primer has the sequence

AGGTATTTCA GCACCGCTGT GTCG SEQ ID NO.: 47

or

GTGAGTGCGG GGTCCAGAGA SEQ ID No.: 48.

SEQ ID No.: 47 amplification primer is complementary to the region of the non-coding strand spanning nucleotides 15-38 of exon 2 of the HLA-F gene sequence (SEQ ID No.: 39). SEQ ID No.: 48 amplification primer is complementary to the region of the non-coding strand spanning nucleotides 1-20 of intron 1 of the HLA-F gene sequence (SEQ ID No.: 49) shown in Fig. 2. It will be appreciated that these amplification primer could be made longer by adding additional complementary bases to either or both ends. The amplification primer might also be made somewhat shorter, for example spanning at least nucleotides 15-20 for SEQ ID No.: 47. The nucleotides complementary to nucleotides 24, 25, 30, 32 and 38 for SEQ ID No.: 47 and 15, 16, 19, and 20 for SEQ ID No.: 48 are also advantageously retained in a locus-specific amplification primer, since these bases are

distinct in the HLA-F sequence from the rest of the genes. In addition, complementary amplification primers which bind to the corresponding portions of the coding strand could be used with a compatible second amplification primer. The use of such longer or shorter amplification primers and of complementary amplification primers is within the scope of the present invention.

Examples of the second amplification primer used in typing the HLA-F gene using the method of the invention have the sequence

GCGTCTCCTT CCCATTCTCC AA SEQ ID NO.: 50

or

CAACCTTGTG CGAGGCCATC CCA SEQ ID No.: 51.

SEQ ID No.: 50 amplification primer binds to the region of the coding strand of exon 3 of the HLA-F gene (SEQ ID No.: 40) spanning nucleotides 243-264. SEQ ID No.: 51 amplification primer binds to the region of the coding strand of intron 3 of the HLA-F gene (SEQ ID No.: 52) spanning nucleotides 46-68. As in the case of the first amplification primer, amplification primers which are a made a few bases longer by virtue of adding additional complementary bases, amplification primers which are a few bases shorter (retaining the 3'-end), and complementary amplification primers may be used in the method of the present invention.

Amplification of the HLA-F gene is performed using the same methodology described for amplification of HLA-A, -B and -C genes, and the same cycle program described for amplification of the HLA-E gene.

Suitable probes for use in hybridization assays of the type of the amplified product made using these amplification primers are listed in Table 1.

For typing the HLA-G gene, the first amplification primer has, for example, the sequence

GGTTCGACAG CGACTCGGCG T SEQ ID NO.: 53

or

CGGCGGGGGC GCAGGACTCG GCA SEQ ID NO.: 54.

SEQ ID NO.: 53 amplification primer is complementary to the region of the non-coding strand spanning nucleotides 103-123 of exon 2 of the HLA-G gene sequence (SEQ ID No.: 39). SEQ ID NO.: 54 amplification primer is complementary to the region of the non-

coding strand spanning nucleotides 56-78 of intron 1 of the HLA-G gene sequence (SEQ ID No.: 55). It will be appreciated that this amplification primer could be made longer by adding additional complementary bases to either or both ends. The amplification primer might also be made somewhat shorter. The nucleotides complementary to nucleotides 119, 120 and 123 for SEQ ID NO.: 53 and 79 and 80 for SEQ ID No.: 54 are advantageously retained in a locus-specific amplification primer, since these bases are distinct in the HLA-G sequence from the rest of the genes. In addition, complementary amplification primers which bind to the corresponding portions of the coding strand could be used with a compatible second amplification primer. The use of such longer or shorter amplification primers and of complementary amplification primers is within the scope of the present invention.

Suitable oligonucleotides for use as the second amplification primer used in typing the HLA-G gene using the method of the invention have the sequence

TCTCCTTCCC GTTCTCCAGG T SEQ ID NO.: 56

or

TCCTCCTCTC CTTGTGCTAG GCCAGGCTG SEQ ID NO.: 57.

SEQ ID NO.: 56 amplification primer binds to the region of the coding strand of exon 3 of the HLA-G gene (SEQ ID No.: 40) spanning nucleotides 241-261. This region is the same in HLA-G, -H, -J, -K, and -L genes, and the same primer can be used as a second primer in each of the amplifications. SEQ ID NO.: 36 amplification primer binds to the region of the coding strand of exon 3 of the HLA-G gene (SEQ ID No.: 40) spanning nucleotides 46-74. Slightly longer, shorter and complementary primers can also be used.

Amplification of the HLA-G gene is performed using the same general methodology described for amplification of HLA-A, -B and -C genes. In this case, however the amplification is performed in a glycerol buffer, rather than a DMSO buffer. Thus, each reaction mixture contains 10 to 100 ng of genomic DNA in a 100 µl volume containing 0.2 mM dNTPs, the two amplification primers at a concentration of 0.2 µM each, 2.5 units of Taq polymerase, 10 mM Tris-HCl (pH 8.8), 1.5 mM $MgCl_2$, 0.001% (w/v) gelatin, 50 mM KCl and 7.5% (w/v) glycerol. The cycle program for amplification in this case is preferably

denaturation	94°C	22 seconds
annealing	60°C	50 seconds

extensions	72°C	30 seconds.

Suitable probes for use in hybridization assays of the type of the amplified product made using these amplification primers are listed in Table 1.

For typing the HLA-H gene, a suitable first amplification primer has the sequence

GAGCCCCGCT TCATCTCCGT C SEQ ID NO.: 58.

This amplification primer is complementary to the region of the non-coding strand spanning nucleotides 54-74 of exon 2 of the HLA-G gene sequence (SEQ ID No.: 39). It will be appreciated that this amplification primer could be made longer by adding additional complementary bases to either or both ends. The amplification primer might also be made somewhat shorter. The nucleotides complementary to nucleotides 69, 71 and 74 for SEQ ID No.: 58 are advantageously retained in a locus-specific amplification primer, since these bases are distinct in the HLA-H sequence from the rest of the genes. In addition, complementary amplification primers which bind to the corresponding portions of the coding strand could be used with a compatible second amplification primer. The use of such longer or shorter amplification primers and of complementary amplification primers is within the scope of the present invention.

The second amplification primer used in typing the HLA-H gene is the same as that used for HLA-G (SEQ ID NO.: 56). Alternative second amplification primers can be made from nucleotides which include nucleotides 485 (G), 486 (A) and 490 (T) in exon 3 or nucleotides 28(G) and 33(G) in intron 3.

Amplification of the HLA-H gene is performed using the same general methodology described for amplification of HLA-A, -B and -C genes in DMSO buffer. The cycle program for amplification in this case is preferably

denaturation	94°C	22 seconds
annealing	58°C	50 seconds
extensions	72°C	30 seconds.

Suitable probes for use in hybridization assays of the type of the amplified product made using these amplification primers are listed in Table 1.

For typing the HLA-J gene, the first amplification primer has the sequence

AGCACCGCCG TTTCCTGGCC G SEQ ID NO.: 59

This amplification primer is complementary to the region of the non-coding strand spanning nucleotides 24-44 of exon 2 of the HLA-G gene sequence (SEQ ID No.: 39). It will be appreciated that this amplification primer could be made longer by adding additional complementary bases to either or both ends. The amplification primer might also be made somewhat shorter. The nucleotides complementary to nucleotides 30, 35, 39, and 44 for SEQ ID NO. 59 are advantageously retained in a locus specific amplification primer, since these bases are distinct in the HLA-J sequence from the rest of the genes. In addition, complementary amplification primers which bind to the corresponding portions of the coding strand could be used with a compatible second amplification primer. The use of such longer or shorter amplification primers and of complementary amplification primers is within the scope of the present invention.

The second amplification primer used in typing the HLA-J gene is the same as that used for HLA-G (SEQ ID NO.: 56).

Amplification of the HLA-J gene is performed using the same methodology described for amplification of the HLA-G gene.

Suitable probes for use in hybridization assays of the type of the amplified product made using these amplification primers are listed in Table 1.

For typing the HLA-K gene, the first amplification primer has the sequence

ACTCCATAAG GTAGTTCAGC ACCGCC SEQ ID NO.: 60

This amplification primer is complementary to the region of the non-coding strand spanning nucleotides 7-32 of exon 2 of the HLA-K gene sequence (SEQ ID No.: 39). It will be appreciated that this amplification primer could be made longer by adding additional complementary bases to either or both ends. The amplification primer might also be made somewhat shorter, for example spanning at least nucleotides 7-15. In addition, complementary amplification primers which bind to the corresponding portions of the coding strand could be used with a compatible second amplification primer. The use of such longer or shorter amplification primers and of complementary amplification primers is within the scope of the present invention.

The second amplification primer used in typing the HLA-K gene is the same as that used for HLA-G (SEQ ID NO.: 56).

Amplification of the HLA-K gene is performed using the same methodology described for amplification of the HLA-G gene.

Suitable probes for use in hybridization assays of the type of the amplified product made using these amplification primers are listed in Table 1.

For typing the HLA-L gene, the first amplification primer has the sequence

GTGCGGTTCG ACAGCGACTC CGT SEQ ID NO.: 61

This amplification primer is complementary to the region of the non-coding strand spanning nucleotides 99-121 of exon 2 of the HLA-L gene sequence (SEQ ID No.: 39). It will be appreciated that this amplification primer could be made longer by adding additional complementary bases to either or both ends. The amplification primer might also be made somewhat shorter. The nucleotides complementary to nucleotides 117 and 121 for SEQ ID No.: 61 are advantageously retained in the locus-specific amplification primer, since these bases are distinct in the HLA-L sequence from the rest of the genes. In addition, complementary amplification primers which bind to the corresponding portions of the coding strand could be used with a compatible second amplification primer. The use of such longer or shorter amplification primers and of complementary amplification primers is within the scope of the present invention.

The second amplification primer used in typing the HLA-L gene is the same as that used for HLA-G (SEQ ID NO.: 56).

Amplification of the HLA-L gene is performed using the same methodology described for amplification of the HLA-G gene.

Suitable probes for use in hybridization assays of the type of the amplified product made using these amplification primers are listed in Table 1.

The primers used in the determination of HLA type in accordance with the invention can be made by any of the methods known in the art, and indeed companies now exist which will make a desired oligonucleotide to order. Examples of suitable synthetic approaches for primers include the phosphoramidite method.

The amplification primers which are themselves an aspect of the invention may be modified using methods known in the art to include a detectable label or a capture moiety such as biotin. For example, a fluorophore can be added to the 5'-terminus of a primer by synthesizing the oligonucleotide with a 5'-aliphatic amino group and then coupling the

amino group to an activated dye precursor. The 3'-terminus of an last oligonucleotide can be labeled using Terminal deoxynucleotidyl transferase to add a single extra fluorescently-labeled nucleotide from a fluorescent dideoxy(NTP) precursor. All necessary reagents for this 3'-labeling procedure are available commercially. (ABI, Boehringer, Clontech). Such labeled primers may be useful as sequencing primers for determining the sequence of the amplified portion of the gene.

Amplification primers in accordance with the invention may be advantageously packaged in kits for the typing of tissue samples. Such kits may contain, for example, at least one pair of amplification primers, including at least one locus-specific amplification primer, effective to amplify at least one HLA Class I gene. In addition the kits may include some or all of the following:

(1) one or more reagents for the amplification of the HLA gene using the primers, e.g., a polymerase enzyme, a buffer, and individual nucleotide bases;

(2) one or more sequencing primers suitable for sequencing exons 2 and 3 of the gene(s) amplified by the primer pair(s), together with optional sequencing reagents such as polymerase;

(3) one or more sequence-specific oligonucleotide probes useful for determining the HLA type of the gene(s) amplified by the primer pair(s); and

(4) reagents for sample preparation. Such kits may also include instructions for carrying out the tissue preparation and typing, containers, dot blot membranes or other solid supports for hybridization assays.

EXAMPLE 1

Genomic DNA was prepared from samples of each of 106 cell lines of the cell panel of the 10th International Histocompatibility Workshop using proteinase K digestion as described by Levine et al., *Tissue Antigens* 44: 174-183 (1994). The name and HLA-A, HLA-B and HLA-C types of each of these cell lines is listed in Fig. 8.

A portion of each prepared sample was amplified using one of the probe combinations described above, i.e,

for HLA-A Seq ID Nos.: 11 and 13;

for HLA-B Seq ID Nos.: 23 and 26; and

for HLA-C Seq ID Nos.: 30 and 29

for HLA-E	Seq ID Nos.: 41 and 44
for HLA-F	Seq ID Nos.: 47 and 50
for HLA-G	Seq ID Nos.: 53 and 56
for HLA-H	Seq ID Nos.: 58 and 56
for HLA-J	Seq ID Nos.: 59 and 56
for HLA-K	Seq ID Nos.: 60 and 56
for HLA-L	Seq ID Nos.: 61 and 56

under the conditions described above. The amplified products were then applied to a positively charged nylon membrane (Boehringer Mannheim, Germany) using an eight channel syringe. After air-drying for one hour, DNA dotted on the membrane was denatured in 0.4 M NaOH. After neutralization, DNA was UV cross-linked to the membrane by exposing it for 5 minutes in a Stratalinker 2400 (Stratagene).

Oligonucleotide probes as shown in Table 1s were 3'-end labeled with digoxigenin-ddUTP (Boehringer Mannheim) in accordance with the manufacturers instructions. The membranes were then hybridized with digoxigenin-ddUTP labeled oligonucleotide probes (1 pmol/ml hybridization solution) of the types for one hour. The hybridization was conducted at 46°C for 15-mer probes and 54°C for 18-mer probes. The membranes were then washed in TMAC at 54°C and 58°C, respectively, for 20 minutes. Washed membranes were treated with anti-digoxigenin Fab antibody conjugated to alkaline phosphatase (Boehringer Mannheim) after treatment with blocking agent in accordance with the manufacturers protocol. The washed and treated membranes were then treated with Lumiphos 480 (Life Codes, Stamford CT) according to the manufacturers instructions and imaged using Kodak X-Omat X-Ray film for 1 to 60 minutes.

A first set of membranes was used to test the locus specificity of the amplification primers of the invention using locus-specific probes. In this set of tests, each membrane had amplifications products for each locus on it as shown in Table 2.

TABLE 2 - PROTOCOL FOR LOCUS SPECIFICITY TEST	
GENE	CELL LINES TESTED
HLA-A	1, 2, 5, 6, 7, 8, 9, 10, 13, 14, 16, 19, 22, 29, 35, 50, 53, 58, 64, 66, 71, 106, 107, NEGATIVE CONTROL
HLA-B	SAME AS HLA-A
HLA-C	SAME AS HLA-A
HLA-E	24, 25, 26, 28, 30, 41, 48, 55
HLA-F	13, 14, 16, 19, 22, 29, 35, 50
HLA-G	SAME AS HLA-F
HLA-H	63, 64, 65, 66, 67, 68, 69, 70
HLA-J	SAME AS HLA-F
HLA-K	7, 13, 16, 19, 22, 29, 35, 50
HLA-L	SAME AS HLA-F

These membranes were hybridized with probes 131R (and HLA-A and HLA-A specific probe); HBB034 (an HLA-B specific probe) HYB035 (HLA-C, G, L and B54 specific); EE2-210 (HLA-E specific); FE2-200 (HLA-F specific) GE3-183 (HLA-G specific) HE3-479 (HLA-H specific); JE3-274 (HLA-J and -K specific); and HLB032 (HLA-L specific).

Probe 131 R showed positive hybridization with all cell lines amplificd with the HLA-A specific probes and all cell lines except cell line 35 and 50 amplified with the HLA-L specific probes; and no positive hybridization results with any other amplification products.

Probe HBB034 showed positive hybridization with all cell lines amplified with the HLA-B specific probes; and no positive hybridization results with any other amplification products.

Probe HYB035 showed positive hybridization with all cell lines except cell lines 35 and 107 amplified with the HLA-C specific probes and all cell lines except cell line 35 amplified with the HLA-G or K specific probes. One false positive hybridization result was noted for cell line 107 amplified with the HLA-B specific probes. This is consistent with published sequence data which shows that the cell line carries the HLA-Cw consensus sequence in the site from which HBB034 was derived.

Probe EE2-210 showed positive hybridization with all cell lines amplified with the HLA-E specific probes; and no positive hybridization results with any other amplification products.

Probe FE2-200 showed positive hybridization with all cell lines amplified with the HLA-F specific probes; and no positive hybridization results with any other amplification products.

Probe GE3-183 showed positive hybridization with all cell lines amplified with the HLA-G specific probes. False positive hybridization results were also obtained for all cell lines amplified with the HLA-L specific probes.

Probe HE3-479 showed positive hybridization with all cell lines amplified with the HLA-H specific probes; and no positive hybridization results with any other amplification products.

Probe JE3-274 showed positive hybridization with all cell lines amplified with the HLA-J and K specific probes; and no positive hybridization results with any other amplification products.

Probe HLB032 showed positive hybridization with all cell lines amplified with the HLA-L specific probes; and no positive hybridization results with any other amplification products. This probe can therefore be used to confirm a positive result obtained using GE3-183.

EXAMPLE 2

A second experiment was performed using membranes prepared in accordance with Example 1, except that the membranes were dotted with the amplification products from all 106 cell lines shown in Fig. 7. The membranes also contained a panel of amplification products from the non-classical genes, as follows:

Gene	Cell Lines
HLA-E	4, 5, 6,
HLA-F	19, 20, 21
HLA-G	33, 34
HLA-H	49, 50, 51
HLA-J	64, 65, 66
HLA-K	79, 80, 81

HLA-L	94, 95, 96

In this experiment, HLA-A specific probes 114 EH and 142 IK were hybridized with membranes dotted with amplification products formed using the HLA-A specific primers plus the non-classical panel; and HLA-B specific probes HYE024, HXC008, HBC009 and HBB055 were hybridized with membranes dotted with amplification products formed using the HLA-B specific primers plus the non-classical panel.

On all of the membranes, a positive hybridization result was obtained for every cell lines which met the known specificity of the probe, as set forth in Table 2. No positive results were detected for cell lines with different allelic types. Thus, locus-specific amplification is achieved using the primer combinations of the invention.

EXAMPLE 3

Three additional membranes were prepared in accordance with the protocols in Example 1. The first membrane was dotted with samples for all 106 cell lines amplified with generic (not locus-specific) HLA amplification primers having the sequence

GGCYGGGGTC ACTCACCG SEQ ID No.: 18

and

TGCAGCGTCT CCTTCCCGTT SEQ ID No.: 84

The second was dotted with samples for all 106 cell lines amplified with the HLA-B specific amplification primers Seq ID Nos.: 3 and 4 of the invention. The third was dotted with samples for all 106 cell lines amplified with the HLA-C specific amplification primers Seq ID Nos.: 5 and 6 of the invention. These membranes were then hybridized with probe 156R which binds to several HLA-B and HLA-C allelic types.

The membrane dotted with generic amplification products revealed no specificity, with all cell lines producing a positive hybridization result. In contrast, with HLA-B amplified fragments, the probe reacted only with amplified products from cell lines 1, 13, 17, 33, 34, 42, 65, 81, 82 and 83, the cell lines which have allelic types recognized by the 156R probe. On the membrane dotted with HLA-C amplified fragments, the probe showed a positive hybridization reaction with all samples carrying Cw1, Cw4, Cw5, Cw8 and Cw14 but not

with those with other HLA-C allelic types. This test further demonstrates the locus specificity of the amplification procedure of the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:
(i) APPLICANT: Yang, Soo Young
Cereb, Nezih
(ii) TITLE OF INVENTION: Methods and Reagents for Typing HLA Class I Genes
(iii) NUMBER OF SEQUENCES: 84
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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette - 3.5 inch, 1.44 Mb storage
(B) COMPUTER: IBM compatible
(C) OPERATING SYSTEM: MS DOS
(D) SOFTWARE: Word Perfect
(vi) CURRENT APPLICATION DATA :
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:
(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
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(2) INFORMATION FOR SEQ ID NO: 1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 130
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: Genomic DNA
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:

(A) ORGANISM: human
(D) OTHER INFORMATION: consensus sequence of intron 1 of the HLA-A gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
GTGAGTGCGG GGTCGKGAGG GAAACSGCCT CTGYGGGGAG AAGCAASGGG
50
CCCKCCYGGC GGGGRCGCAR GACCSGGGDA GCCGCGCCKG GASGAGGGTC
100 GGKYRGRTCT CAGCCWCTSC TCGYCCCCAG 130

(2) INFORMATION FOR SEQ ID NO: 2:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 242
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: Genomic DNA
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: consensus sequence of intron 2 of the HLA-A gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:
GTGAGTGACC CCRGCCSGGG GCGCAGGTCA SGACCYCTCA TCCCCCACGG
50 ACGGGCCRGG TSCRCCCACA GTCTCCGGGT CCGAGATCCR CCCCGAAGCC
100
GCGGGACYCC GAGACCCTTG HCCCGGGAGA GGCCCAGGCG CCTTWACCCG
150
GTTTCATTTT CAGTTTAGGC CAAAAATYCC CCCRGGTTGG TCGGGGCBGG
200
RCRGGGCTYG GGGGACYGGG CTGACCKYGG GGTCSGGGCC AG 242

(2) INFORMATION FOR SEQ ID NO: 3:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 650
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: Genomic DNA
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: consensus sequence of intron 3 of the HLA-A gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GTACCAGGGG CCACRGRGCG CCTMCCTGAT CGCCTRTAGR TCTCCCGGGC
50 TGGCCTCCCA CAAGGAGGGG AGACAWTTGG GACCAACACT
AGAATATCRC 100 CCTCCCTCTG GTCCTGAGGG AGAGGAMTCC
TCCTGGGTTT CCAGATCCTG 150
TACCAGAGAG TGACTCTGAG GTTCCGCCCT GCTCTSTGAC WCAATTAAGG
200 GATAAAATCT CTGAMGGART GACGGDAAGA CGATCCCTCG
AATACTGATG 300 ASTGGTTCCC TTTGACACAC ACMGGCAGSA
GCCTTGGGMC CGTGACTTTT 350
CCTCTCAGGC CTTGTTCTCT GCTTCACACT CAATGTGTGT GGGGGTCTGA 400
GTCCAGCACT TCTGAGTCYY TCAGCCTCCA CTCAGGTCAG GACCAGAAGT
450 CGCTGTTCCC TYYTCAGGGA MTAGAATTTT CCACGGAATA
GGAGATTATC 500
CCAGGTGCCT GTGTCCAGGC TGGTGTCTGG GTTCTGTGCT CYCTTCCCCA
550 TCCCRGGTGT SCTGTCCATT CTCAAGATRG SCACATGYRT
GCTGGWGGAG 600 TGTCCCATKA CAGATRCMMA ATGCCTGMAT
KWTCTGACTC TTCCYGWCAG 650

(2) INFORMATION FOR SEQ ID NO: 4:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 129
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: Genomic DNA
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: consensus sequence of intron 1 of the HLA-B gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
GTGAGTGCGG GRTCGGSAGG GAAATGGCCT CTGYVGGGAG GAGMGAGGGG
50 ACCTCAGGCG GGGGCGCAGG ACCYGRGGAG CCGCGCCGGG
AGGAGGGTCK 100 GGCGGGTYTC AGCYCCTCCT BRCCCCCAG
129

(2) INFORMATION FOR SEQ ID NO: 5:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 252
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: Genomic DNA
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: consensus sequence of intron 2 of the HLA-B gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:
GTGAGTGACC CCGGCCYGGG GCGSAGGTCA CGACTCCCCA TCCCCCACGK
50 ACGGBCCGGG TCGCCCCGAG TCTCCGGGTC CGAGATCCRM
CYCCCTGAGG 100 CYGSGGGAMC CGCCCAKACC CTCGACCGGM
GAGAGCCSCA GGCGCGTTTA 150
CCCGGTTTCA TTTTCAGTTG AGGCCAAAAA TCCCCGCGGG TTGGKCRGGG
200 CGGGGCGGGG CGGGGCTCGG GGGGACKGKG CTGWCCGCGG
GGBSKGGKCC 250
AG 25?

(2) INFORMATION FOR SEQ ID NO: 6:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 575
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: Genomic DNA
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: consensus sequence of intron 3 of the HLA-B gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:
GTACCAGGGG CAGTGGGGAG CCTBCCCCAT CTCCTATAGG TCGSCGGGGA
50 TGGSCTCCMA CGAGAAGARG AGGAAAATGG GATCAGCGCT
AGAATGTCGC 100 CCTCCCTTGA ATGGAGAATG GCATGAGTTT
TCCTGAGTTT CCTCTGAGGG 150
CCCCCTCTTC TCTCTAGGAC AATTARGGRA TGACGTCTCT GAGGAAATGG
200 AGGGGAAGWC AGYCCCTAGR ATASTGATCA GGGGTCCYCT
TTGACCCCTG 250 CAGCAGCCTT GGGAACCRTG ACTTTTCYTC
TCAGRCCTTG TTCTCTGCCT 300
CACACTCAGT GTGTTTGGGG CTCTGATTCC AGYACTTCTG AGTCACTTTA
350 CCTCCACTCA GATCRGGAGC AGAAGTCYCT GTTCCCCGCT
CAGAGACTCG 400 AACTTTCCAA TGAATAGGAG ATTATCCCAG
GTGCCTGCRT CCAGGCTGGT 450
GTCTGGGTTC TGTGYCCCTT CCCCACMCCA GGTGTCCTGY CCATTCTCAG
500 KCTGGTCACA TGGGTGGTCC TAGGGTGTSC CATGARAGAT
GCMAAGCGCC 550 TGWAWTTTCT GACTCTTCCC ATCAG 575

(2) INFORMATION FOR SEQ ID NO: 7:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 130
(B) TYPE: nucleic acid

(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: Genomic DNA
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: consensus sequence of intron 1 of the HLA-C gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:
GTGAGTGCGR GGTTRGGAGG GAADCGGCCT CTGSGGAGAG GARCGAGGKG
50 CCCKCCCGGC GAGGGCGCAG GACCCGGGGA GCCGCGCAGG
GAGGWGGGTC 100 GGGCGGGTCT CAGCCMCTCC TCKYCCCCAG
130

(2) INFORMATION FOR SEQ ID NO: 8:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 252
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: Genomic DNA
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: consensus sequence of intron 2 of the HLA-C gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:
GTGAGTGACC CCRGCCCGGG GCGCAGGTCA CGACCCCCCC YCATCCCCCA
50 CGGACGGCCC GGGTCGCCCC RAGTCTCCSS GTCTGAGATC CACCCCAAGG
100 TGGATCTGCG GAACCCGCCC AGACCCTCGA CCGGAGAGAG
CCCYAGTCRC 150
CTTTACCCGG TTTCATTTTC RGTTTAGGCC AAAAATCCCC GCSGKTTGGT 200
CGGGRCKGGG GCGGGGCTCG SGGGACKGKG YTGACCRCGG GGGCGGSGCC
250
AG 252

(2) INFORMATION FOR SEQ ID NO: 9:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 587
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE:Genomic DNA
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: consensus sequence of intron 3 of the HLA-C gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:
GTACCAGGGG CAGTGGGGAG CCTTCCCCAT CTCCYRTAGA TCTCCCGGSA
50
TGGCCTCCCA CGAGGAGGGG AGGAAAATGG GATCAGCGCT RGAATATCGC
100
CCTCCCTTGA ATGGAGAATG GSATGAGTTT TCCYGAGTTT CYTCTGARGG
150
CCCCSTCTGC TCTCTAGGAC AATTAAGGGA TGAAGTCYYT GAGGAAATGG
200
AGGGGAAGAC AGTCCCTRGA ATACTGATCA GGGGTCYCCT TTGACCACTT
250
TGACCACTGC RGCAGCTGTG GTCAGGCTGC TGACCTTTCT CTCAGGCCTT
300
GTTCTCTGCC TCAYRYTCAA TGTGTYTRAA GGTTTGATTC CAGCTTTTCT 350
GAGTYCTKCR GCCTCCACTC AGGTCAGGAC CAGAAGTCGC TGTTCCTCCC
400
TCAGAGACTA GAACTTTCCA AWGAATAGGA GATTATCCCA GGTSCCTGTG
450
TCCAGGCTGG CGTCTGGGTT CTGTGCCSCC TTCCCYACCC CAGGTGTCCT 500
GTCCRTTCTC AGGATRGTCA CATGGSCRCT GYTGGAGTGT CSCAAGAGAG
550
AWRCAAAGTG TCTGAATTTT CTGACTCTTC CCGTCAG 587

(2) INFORMATION FOR SEQ ID NO: 10:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: intron 1 primer for locus specific amplification of exons 2 and 3 of HLA-A gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:
GGCCTCTGYG GGGAGAAGCA A 21

(2) INFORMATION FOR SEQ ID NO: 11:
(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: intron 1 primer for locus specific amplification of exons 2 and 3 of HLA-A gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:
GAAACSGCCT CTGYGGGGAG AAGCC 25

(2) INFORMATION FOR SEQ ID NO: 12:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: intron 3 primer for locus specific amplification of exons 2 and 3 of HLA-A gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:
CGGGAGATCT AYAGGCGATC AGG 23

(2) INFORMATION FOR SEQ ID NO: 13:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: intron 3 primer for locus specific amplification of exons 2 and 3 of HLA-A gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:
TGTTGGTCCC AATTGTCTCC CCTC 24

(2) INFORMATION FOR SEQ ID NO: 14:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: intron 3 primer for locus specific amplification of exons 2 and 3 of HLA-A gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:
AGGATTCCTC TCCCTCAGGA CCAG 24

(2) INFORMATION FOR SEQ ID NO: 15:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: sequencing primer for exon 2 of HLA-A, -B or -C genes
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:
GGGTCKGKYR GRTYTCAGC 19

(2) INFORMATION FOR SEQ ID NO: 16:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no

(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: sequencing primer for exon 2 of HLA-A, -B or -C genes
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:
CGCSCMKGGA SGWGGGTC 18

(2) INFORMATION FOR SEQ ID NO: 17:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: sequencing primer for exon 2 of HLA-A, -B or -C genes
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:
TCYCACTCCA TGAGGTATTT C 21

(2) INFORMATION FOR SEQ ID NO: 18:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: sequencing primer for exon 2 of HLA-A, -B or -C genes
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:
GGCYGGGGTC ACTCACCG 18

(2) INFORMATION FOR SEQ ID NO: 19:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17
(B) TYPE: nucleic acid

(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: sequencing primer for exon 2 of HLA-A, -B or -C genes
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:
GTCSTGACCT SCGCCCC 17

(2) INFORMATION FOR SEQ ID NO: 20:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: sequencing primer for exon 3 of HLA-A, -B or -C genes
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:
GCGGGRCGGG GCTCGGGGG 19

(2) INFORMATION FOR SEQ ID NO: 21:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: sequencing primer for exon 3 of HLA-A, -B or -C genes
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:
ATYCCCSCRG KTTGGTC 17

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: sequencing primer for exon 3 of HLA-A, -B or -C genes
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:
CCCYRYKGCC CCTGGTAC 18

(2) INFORMATION FOR SEQ ID NO: 23:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: locus specific amplification primer for HLA-B gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:
GGGAGGAGCG AGGGGACCSC AG 22

(2) INFORMATION FOR SEQ ID NO: 24:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: locus specific amplification primer for HLA-B gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CGGGGGCGCA GGACCCGG 18

(2) INFORMATION FOR SEQ ID NO: 25:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: locus specific amplification primer for HLA-B gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:
GGCGGGGGCG CAGGACCTGA 20

(2) INFORMATION FOR SEQ ID NO: 26:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: locus specific amplification primer for HLA-B gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:
GGAGGCCATC CCCGGCGACC T 21

(2) INFORMATION FOR SEQ ID NO: 27:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: locus specific amplification primer for HLA-B gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:
GGAGGCCATC CCCGGCGACC TAT 23

(2) INFORMATION FOR SEQ ID NO: 28:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: locus specific amplification primer for HLA-B gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:
CTCAGGAAAA CTCATSCCAT TCTCCATTC AAG 33

(2) INFORMATION FOR SEQ ID NO: 29:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: amplification primer for HLA-B or HLA-C gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:
GGAGATGGGG AAGGCTCCCC ACT 23

(2) INFORMATION FOR SEQ ID NO: 30:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: locus specific amplification primer for HLA-C gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:
AGCGAGGXGC CCGCCCGGCG A 21

(2) INFORMATION FOR SEQ ID NO: 31:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: locus specific amplification primer for HLA-C gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:
GAGGGAAACG GCCTCTGCGG A 21

(2) INFORMATION FOR SEQ ID NO: 32:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: locus specific amplification primer for HLA-C gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:
GAGGGGCCCG CCCGGCGA 18

(2) INFORMATION FOR SEQ ID NO: 33:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double

(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: locus specific amplification primer for HLA-C gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:
GACCCGGGGA GCCGCGCA 18

(2) INFORMATION FOR SEQ ID NO: 34:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: locus specific amplification primer for HLA-C gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:
CGCTGATCCC ATTTTCCTCC CCTC 24

(2) INFORMATION FOR SEQ ID NO: 35:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: locus specific amplification primer for HLA-C gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:
CTCAGGAAAA CTCATSCCAT TCTCCATTCA AG 32

(2) INFORMATION FOR SEQ ID NO: 36:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27
(B) TYPE: nucleic acid

(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: locus specific amplification primer for HLA-C gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:
ACCACAGCTG CTGCAGTGGT CAAAGTG 27

(2) INFORMATION FOR SEQ ID NO: 37:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: locus specific amplification primer for HLA-C gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:
GAGGAAAGGT CAGCAGCCTG ACCACA 26

(2) INFORMATION FOR SEQ ID NO: 38:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: locus specific amplification primer for HLA-C gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:
GACTCAGAAA AGCTGGAATC AAACCTT 27

(2) INFORMATION FOR SEQ ID NO: 39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 270
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: consensus sequence of exon 2 of the nonclassical genes
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:
GCTCCCACTC CATGAGGTAT TTCTACACCT CCGTGTCCCG GCCCGGCCGC 50
GGGGAGCCCC GCTTCATCGC AGTGGGCTAC GTGGACGACA CGCAGTTCGT
100
GCGGTTCGAC AGCGACGCCG CGAGTCCGAG GATGGAGCCG CGGGCGCCGT
150
GGATAGAGCA GGAGGGGCCG GAGTATTGGG ACCGGGAGAC ACAGAACTTC
200
AAGGCCCACA CACAGACTGA CCGAGAGAAC CTGCGGAACC TGCGCGGCTA
250
CTACAACCAG AGCGAGGCCG 270

(2) INFORMATION FOR SEQ ID NO: 40:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 276
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: consensus sequence of exon 3 of the nonclassical HLA genes
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:
GGTCTCACAC CCTCCAGAGG ATGTATGGCT GCGACGTGGG GCCGGACGGG
50
CGCCTCCTCC GCGGGTATAA CCAGTACGCC TACGACGGCA AGGATTACAT
100
CGCCCTGAAC GAGGACCTGC GCTCCTGGAC CGCGGCGGAC ACGGCGGCTC
150

AGATCACCCA GCGCAAGTGG GAGGCGGCCC GTGTGGCGGA GCAGCTGAGA
200
GCCTACCTGG AGGGCACGTG CGTGGAGTGG CTCCGCAGAT ACCTGGAGAA
250
CGGGAAGGAG ACGCTGCAGC GCGCGG 276

(2) INFORMATION FOR SEQ ID NO: 41:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: locus specific amplification primer for HLA-E gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:
CACTCCTTGA AGTATTTCCA CACT 24

(2) INFORMATION FOR SEQ ID NO: 42:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: locus specific amplification primer for HLA-E gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:
TGGAAACGGC CTCTACCGGG AGTAGAG 27

(2) INFORMATION FOR SEQ ID NO: 43:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:130
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double

(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: consensus sequence of intron 1 of the HLA-E gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:
GTGAGTGCGG GGTCGGGATG GAAACGGCCT CTACCGGGAG TAGAGAGGGG
50
CCGGCCCGGC GGGGGCGAAG GACTCGGGGA GCCGCGCCGG GAGGAGGGTC
100
GGGCCGATCT CAGCCCCTCC TCGCCCCCAG 130

(2) INFORMATION FOR SEQ ID NO: 44:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: locus specific amplification primer for HLA-E gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:
TCTCCTTCCC CTTCTCCAGG TATT 24

(2) INFORMATION FOR SEQ ID NO: 45:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: locus specific amplification primer for HLA-E gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

CACAGTCCTA GCCCAAGAAG GAGATGGGAG AGTA 34

(2) INFORMATION FOR SEQ ID NO: 46:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 621
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: consensus sequence of intron 3 of the HLA-E gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:
GTAAGAGGGT CCACAGGGCT ACTCTCCCAT CTCCTTCTTG GGCTAGGACT
50
GTGCCCACAG CTGACAGACC TCAAACAGTA GAAGAAACAG GGATGGAGGC
100 CAGAATACCA CTCCTCCCTT GGATCAGGAG AGGGAGCTGT
CACCTGAGGT 150 ACAGGAGATC CTATACCACA GAGTGACTCT
CTTAAAGGGC CAGACCTCTC 200 TCAGGGGCAA TTAAGGAATC
TAGTCTCGCT GGAGATTCCA TCCTTCAGAT 250 GAACTGATGA
GCAGTTCTCT TTGACTCCCA GTATTAGGAA TCACGGGGGA 300
GTTTCTCTCG TGCCTGATTC TCAGCCCCAC ACCAAGAGTT TTTGGAGGTC 350
TGACTCCAGC TTTTCTCAGT CACTCAGCAT CCACACAGGC CAGGACCAGA
400 AATCCCTTTT CACCTTCTAC CCTGGGCTAG CTCATCCCGA TTCTAGAACT
450 TTCCAAGGAA TAAGAGGCTA TCCCAGATCC CTAAGTCCAG
GCTGGTGTCA 500 AGGTTTTGTC CTCTTCTCCT ACTATAATTG TCCTCTTCCT
TCTCAGGATG 550 GTCACATGGG TGCTGCTGGA GTGTCCCATG
AGAGATACAA AGTGCCTGAA 600 TTTTCTGACT CTTCCCCTCA G
621

(2) INFORMATION FOR SEQ ID NO: 47:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: locus specific amplification primer for HLA-F gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:
AGGTATTTCA GCACCGCTGT GTCG 24

(2) INFORMATION FOR SEQ ID NO: 48:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: locus specific amplification primer for HLA-F gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:
GTGAGTGCGG GGTCCAGAGA 20

(2) INFORMATION FOR SEQ ID NO: 49:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:130
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: consensus sequence of intron 1 of the HLA-F gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:
GTGAGTGCGG GGTCCAGAGA GAAACGGCCT CTGTGGGGAG GAGTGAGGGG
50
CCCGCCCGGT GGGGGCGCAG GACTCAGGGA GCCGCGCCCG GAGGAGGGTC
100
TGGCGGGTCT CAGCCCCTCC TCGCCCCCAG 130

(2) INFORMATION FOR SEQ ID NO: 50:
(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: locus specific amplification primer for HLA-F gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:
GCGTCTCCTT CCCATTCTCC AA 22

(2) INFORMATION FOR SEQ ID NO: 51:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: locus specific amplification primer for HLA-F gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:
CAACCTTGTG CGAGGCCATC CCA 23

(2) INFORMATION FOR SEQ ID NO: 52:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: consensus sequence of intron 3 of the HLA-F gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

GTACCAGGGG CCATGGGCGC CTTCCCTATC TCCTGTAGAT CTCTTGGGAT 50

GGCCTCGCAC AAGGTTGGGA GGAAAGTGGG CCCAATGCTA GGATATCGCC
100 CTCCCTCTAG TCCTGAGTAG GAAGAATCTT CCTGGCTTTT CGAGATCCGG
150 TACCAGAGAG TGATTGTGAG AGTCCGCCCT GCTCTCTTGG
ACAATTAAGG 200 GATGAAATGG AGGAGGACAG TCCCTGGTCC
CCTTTGAGCC TCCAACAGCT 250 GCCGTGACTT TTCTCTCAGG TTTTGTCTCT
GCCTCACACT CAATGTGTTT 300
GGGGCTCTGA TTCCAGTCCC TCGCCCTCCA CTTAGTCAGG CCAGAAGTCC
350
CTGCTCCCGC TCAGAGACTC GAACTTTCCA AGGAATAGGA GATTTTCCCA
400
GGTGTCTGTG TCCAGCCTGG TGTCTGGGTT CTGTGCTCCC TTCCCCACCC 450
CAGGTGTCCT GTCCAGTCTC AGGTTGGTCA CATGGGTGCT GCTGGGGTTT
500
CCCATGAGGA GTGCAAAGTG CCTGAATTTT CTGACTCTTC TCAG 544

(2) INFORMATION FOR SEQ ID NO: 53:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: locus specific amplification primer for HLA-G gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:
GGTTCGACAG CGACTCGGCG T 21

(2) INFORMATION FOR SEQ ID NO: 54:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: locus specific amplification primer for HLA-G gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:
CGGCGGGGGC GCAGGACTCG GCA 23

(2) INFORMATION FOR SEQ ID NO: 55:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 129
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: consensus sequence of intron 1 of the HLA-G gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:
GTGAGTGCGG GGTCAGGAGG GAAACGGCCC CTGCGCGGAG GAGGGAGGGG
50
CCCCCCCGGC GGGGGCGCAG GACTCGGCAG CCGCGCCGGG AGGAGGGTCG
100
GGCGGGTCTC AACCCCTCCT CGCCCCCAG 129

(2) INFORMATION FOR SEQ ID NO: 56:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: locus specific amplification primer for HLA-G gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:
TCTCCTTCCC GTTCTCCAGG T 21

(2) INFORMATION FOR SEQ ID NO: 57:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: locus specific amplification primer for HLA-G gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:
TCCTCCTCTC CTTGTGCTAG GCCAGGCTG 27

(2) INFORMATION FOR SEQ ID NO: 58:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: locus specific amplification primer for HLA-H gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:
GAGCCCCGCT TCATCTCCGT C 21

(2) INFORMATION FOR SEQ ID NO: 59:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: locus specific amplification primer for HLA-J gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:
AGCACCGCCG TTTCCTGGCC G 21

(2) INFORMATION FOR SEQ ID NO: 60:
(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: locus specific amplification primer for HLA-K gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:
ACTCCATAAG GTAGTTCAGC ACCGCC 26

(2) INFORMATION FOR SEQ ID NO: 61:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: locus specific amplification primer for HLA-L gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:
GTGCGGTTCG ACAGCGACTC CGT 23

(2) INFORMATION FOR SEQ ID NO: 62:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: hybridization probe 131R for typing of HLA Class I genes
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

CGCTCTTGGA CCGCG 15

(2) INFORMATION FOR SEQ ID NO: 63:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: hybridization probe HBB034 for typing of HLA Class I genes
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:
GTTCGTGAGG TTCGACAGC 19

(2) INFORMATION FOR SEQ ID NO: 64:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: hybridization probe HYB035 for typing of HLA Class I genes
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:
CGCCGTGGGT GGAGCAGGA 19

(2) INFORMATION FOR SEQ ID NO: 65:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: hybridization probe EE2-210 for typing of HLA Class I genes
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:
GCACAGACAC GGAACACC 18

(2) INFORMATION FOR SEQ ID NO: 66:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: hybridization probe FE2-200 for typing of HLA Class I genes
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:
GTCTGTGCGT TGGCCTTG 18

(2) INFORMATION FOR SEQ ID NO: 67:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: hybridization probe GE2-183 for typing of HLA Class I genes
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:
GAGGAGACAC GGAACACC 18

(2) INFORMATION FOR SEQ ID NO: 68:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: hybridization probe HE3-479 for typing of HLA Class I genes
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:
TCCACGAACT CGCCCTCC 18

(2) INFORMATION FOR SEQ ID NO: 69:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: hybridization probe JE3-274 for typing of HLA Class I genes
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:
TTCCCTGGAG GATGTGAT 18

(2) INFORMATION FOR SEQ ID NO: 70:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:

(A) ORGANISM: human
(D) OTHER INFORMATION: hybridization probe HLB-032 for typing of HLA Class I genes
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:
CAGCGACTCC GTGAGTCCG 19

(2) INFORMATION FOR SEQ ID NO: 71:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: hybridization probe 142IK for typing of HLA Class I genes
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:
CAGATCACCA AGCGC 15

(2) INFORMATION FOR SEQ ID NO: 72:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: hybridization probe 114EH for typing of HLA Class I genes
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:
TATGAACAGC ACGCC 15

(2) INFORMATION FOR SEQ ID NO: 73:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19
(B) TYPE: nucleic acid

(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: hybridization probe HXC008 for typing of HLA Class I genes
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:
CTGCGGATCG CGCTCCGCT 19

(2) INFORMATION FOR SEQ ID NO: 74:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: hybridization probe HBB055 for typing of HLA Class I genes
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:
CCGCGAGTCC GAGGATGGC 19

(2) INFORMATION FOR SEQ ID NO: 75:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: hybridization probe HBC009 for typing of HLA Class I genes
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

CTGCGGACCC TGCTCCGCT 19

(2) INFORMATION FOR SEQ ID NO: 76:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: hybridization probe HYE024 for typing of HLA Class I genes
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:
GGACCTGCGC TCCTGGACC 19

(2) INFORMATION FOR SEQ ID NO: 77:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: hybridization probe HBD080 for typing of HLA Class I genes
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:
CGGGTACCAC CAGGACGCC 19

(2) INFORMATION FOR SEQ ID NO: 78:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes

(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: hybridization probe HBD083 for typing of HLA Class I genes
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:
CGGGTATGAC CAGGACGCC

(2) INFORMATION FOR SEQ ID NO: 79:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: hybridization probe HBD086 for typing of HLA Class I genes
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:
CGGGTATAAC CAGTTAGCC 19

(2) INFORMATION FOR SEQ ID NO: 80:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: hybridization probe HBF094 for typing of HLA Class I genes
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:
GACAAGCTGG AGCGCGCTG 19

(2) INFORMATION FOR SEQ ID NO: 81:
(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: hybridization probe HBC065 for typing of HLA Class I genes
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:
GAAGTACAAG CGCCAGGCA 19

(2) INFORMATION FOR SEQ ID NO: 82:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: hybridization probe HBC066 for typing of HLA Class I genes
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:
GAACATGAAG GCCTCCGCG 19

(2) INFORMATION FOR SEQ ID NO: 83:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: yes
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: hybridization probe 156R for typing of HLA Class I genes
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:
GCGGAGCAGC GGAGAGCC 18

(2) INFORMATION FOR SEQ ID NO: 84:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL:no
(iv) ANTI-SENSE: no
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(D) OTHER INFORMATION: generic amplification primer for HLA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:
TGCAGCGTCT CCTTCCCGTT 20

CLAIMS

1. A method for testing a tissue sample to determine the allelic type of an HLA Class I gene in the sample, said HLA Class I gene being selected from among HLA-A, HLA-B and HLA-C genes comprising the steps of

(a) treating the tissue sample to obtain nucleic acid polymers suitable for amplification;

(b) combining the nucleic acid polymers with a first primer which hybridizes with a portion of intron 1 or intro 3 of the HLA Class I gene, and a second primer which hybridizes with a different portion of the HLA Class I gene under conditions suitable for amplification to obtain an amplified product; and

(c) evaluating the amplified product to determine the allelic type of the HLA-Class I gene.

2. The method of claim 1, wherein at least one of the first primer and the second primer specifically hybridizes with the selected HLA Class I gene to provide locus-specific amplification.

3. The method of claim 1 or 2, wherein the first primer hybridizes with intron 1 and the second primer hybridizes with intron 3 of the selected HLA Class I gene.

4. The method of any of claims 1 to 3, wherein the HLA Class I gene is an HLA-A gene, and the first primer is an oligonucleotide which is complementary to or has the same sequence as a portion of SEQ ID No.: 1.

5. The method of any of claims 1 to 3, wherein the HLA Class I gene is an HLA-A gene, and the first primer is an oligonucleotide which is complementary to or has the same sequence as a portion of SEQ ID No.: 3.

6. The method of any of claims 1 to 3, wherein the HLA Class I gene is an HLA-B gene, and the first primer is an oligonucleotide which is complementary to or has the same sequence as a portion of SEQ ID No.: 4.

7. The method of any of claims 1 to 3, wherein the HLA Class I gene is an HLA-B gene, and the first primer is an oligonucleotide which is complementary to or has the same sequence as a portion of SEQ ID No.: 6.

8. The method of any of claims 1 to 3, wherein the HLA Class I gene is an HLA-C gene, and the first primer is an oligonucleotide which is complementary to or has the same sequence as a portion of SEQ ID No.: 7.

9. The method of any of claims 1 to 3, wherein the HLA Class I gene is an HLA-C gene, and the first primer is an oligonucleotide which is complementary to or has the same sequence as a portion of SEQ ID No.: 9.

10. The method of any of claims 1 to 9, wherein the amplified product is evaluated using sequence specific oligonucleotide probes which hybridize selectively to known alleles of the HLA gene.

11. The method of any of claims 1 to 9, wherein the amplified product is evaluated by direct sequencing.

12. The method of claim 11, wherein the amplified product is sequenced using a sequencing primer which hybridizes to all of the classical HLA Class I genes.

13. The method of any of claims 1 to 3, wherein the first amplification primer is any of the primers identified by SEQ ID Nos.: 10-14 and 23-38.

14. The method of any of claims 1 to 13, further comprising the step of testing a portion of the nucleic acid polymers from the sample to determine the type of at least one non-classical HLA Class I genes.

15. The method of claim 14, wherein the step of testing the portion of the nucleic acid polymers includes the steps of

combining the portion of the nucleic acid polymers with a non-classical primer which specifically hybridizes with a portion of the non-classical HLA Class I gene, and a primer which hybridizes with a different portion of the non-classical HLA Class I gene under conditions suitable for amplification to obtain an amplified non-classical product; and

evaluating the amplified non-classical product to determine the allelic type of the non-classical HLA-Class I gene.

16. The method of claim 15, wherein the non-classical primer is any of the primers identified by SEQ ID Nos.: 41, 42, 45, 47, 48, 50, 51, 53, 54, and 56 to 61.

17. An oligonucleotide having a length of from 10 to 45 bases which hybridizes with or has the same sequence as a continuous portion of intron 1, intron 2 or intron 3 of a classical HLA Class I gene.

18. The oligonucleotide of claim 17, wherein the oligonucleotide is a locus-specific probe which specifically hybridizes with or has the same sequence as a portion of intron 1 of one and only one HLA Class I gene.

19. The oligonucleotide of claim 17 or 18, wherein the oligonucleotide specifically hybridizes with or has the same sequence as a portion SEQ ID No.: 1.

20. The oligonucleotide of claim 17 or 18, wherein the oligonucleotide specifically hybridizes with or has the same sequence as a portion SEQ ID No.: 4.

21. The oligonucleotide of claim 17 or 18, wherein the oligonucleotide specifically hybridizes with or has the same sequence as a portion SEQ ID No.: 7.

22. The oligonucleotide of claim 17, wherein the oligonucleotide is a locus-specific probe which specifically hybridizes with or has the same sequence as a portion of intron 3 of one and only one HLA Class I gene.

23. The oligonucleotide of claim 17 or 22, wherein the oligonucleotide specifically hybridizes with or has the same sequence as a portion SEQ ID No.: 3.

24. The oligonucleotide of claim 17 or 22, wherein the oligonucleotide specifically hybridizes with or has the same sequence as a portion SEQ ID No.: 6.

25. The oligonucleotide of claim 17 or 22, wherein the oligonucleotide specifically hybridizes with or has the same sequence as a portion SEQ ID No.: 9.

26. The oligonucleotide of claim 17, wherein the oligonucleotide specifically hybridizes with or has the same sequence as a portion SEQ ID No.: 2.

27. The oligonucleotide of claim 17 or 26, wherein the oligonucleotide specifically hybridizes with or has the same sequence as a portion SEQ ID No.: 5.

28. The oligonucleotide of claim 17 or 26, wherein the oligonucleotide specifically hybridizes with or has the same sequence as a portion SEQ ID No.: 8.

29. The oligonucleotide of claim 17, wherein the oligonucleotide has the sequence given by any of SEQ ID Nos.: 10-14, 23-38, 41, 42, 45, 47, 48, 50, 51, 53, 54, and 56 to 61.

30. A method for preparing an amplification primer pair for locus-specific amplification of exons 2 and 3 of a selected classical HLA Class I gene comprising the steps of:

(a) evaluating the aligned sequences of intron 1 of the classical HLA Class I gene to select an intron 1 sequence of from 10 to 40 bases which differs in the selected gene from unselected classical HLA Class I genes;

(b) scanning the known sequences of the selected and unselected classical HLA Class I genes to determine if the selected intron 1 sequence is repeated elsewhere within the genes and selecting a new intron 1 sequence if repetition is found;

(c) evaluating the aligned sequences of intron 3 of the classical HLA Class I gene to select an intron 3 sequence of from 10 to 40 bases which differs in the selected gene from unselected classical HLA Class I genes;

(d) scanning the known sequences of the selected and unselected classical HLA Class I genes to determine if the selected intron 3 sequence is repeated elsewhere within these genes and selecting a new intron 3 sequence if repetition is found; and

(e) synthesizing a pair of primers having the sequences of the selected intron 1 and intron 3 sequences.

31. The method of claim 30, further comprising the step of performing a test amplification using the synthesized primers and testing the amplification products with sequence specific probes to confirm locus specificity.

32. A kit for testing a tissue sample to determine the allelic type of an HLA Class I gene in the sample comprising, in packaged combination, at least one pair of amplification primers, said pair of amplification primers including at least a first primer which hybridizes with a portion of intron 1 or intron 3 of the HLA Class I gene.

33. The kit of claim 32, wherein the first primer is a locus-specific primer which specifically hybridizes with one and only one of the HLA Class I genes.

34. The kit of claim 32 or 33, wherein the HLA Class I gene is an HLA-A gene, and the first primer specifically hybridizes with or is the same as a continuous portion of SEQ ID NO.: 1, 2 or 3.

35. The kit of claim 32 or 33, wherein the HLA Class I gene is an HLA-B gene, and the first primer specifically hybridizes with or is the same as a continuous portion of SEQ ID NO:: 4, 5 or 6.

36. The kit of claim 32 or 33, wherein the HLA Class I gene is an HLA-C gene, and the first primer specifically hybridizes with or is the same as a continuous portion of SEQ ID NO.: 7, 8 or 9.

37. The kit of any of claims 32 to 36, further comprising at least one separate container containing a sequence-specific oligonucleotide probe which hybridizes selectively to a known allele of the HLA gene.

38. A method for testing a tissue sample to determine the allelic type of an HLA Class I gene in the sample, said HLA Class I gene being selected from among the non-classical HLA genes comprising the steps of

(a) treating the tissue sample to obtain nucleic acid polymers suitable for amplification;

(b) combining the nucleic acid polymers with a locus-specific first primer which specifically hybridizes with a portion of the non-classical HLA Class I gene, and a second primer which hybridizes with a different portion of the non-classical HLA Class I gene under conditions suitable for amplification to obtain an amplified non-classical product; and

(c) evaluating the amplified non-classical product to determine the allelic type of the non-classical HLA Class I gene.

39. The method of claim 38, wherein the locus-specific first primer is any of the primers identified by SEQ ID Nos.: 41, 42, 45, 47, 48, 50, 51, 53, 54, and 57 to 61.

40. The method of claim 38 or 39, wherein the second primer has the sequence given by SEQ ID No.: 56.

EXON-INTRON ORGANIZATION OF HLA CLASS I GENES

Fig. 1

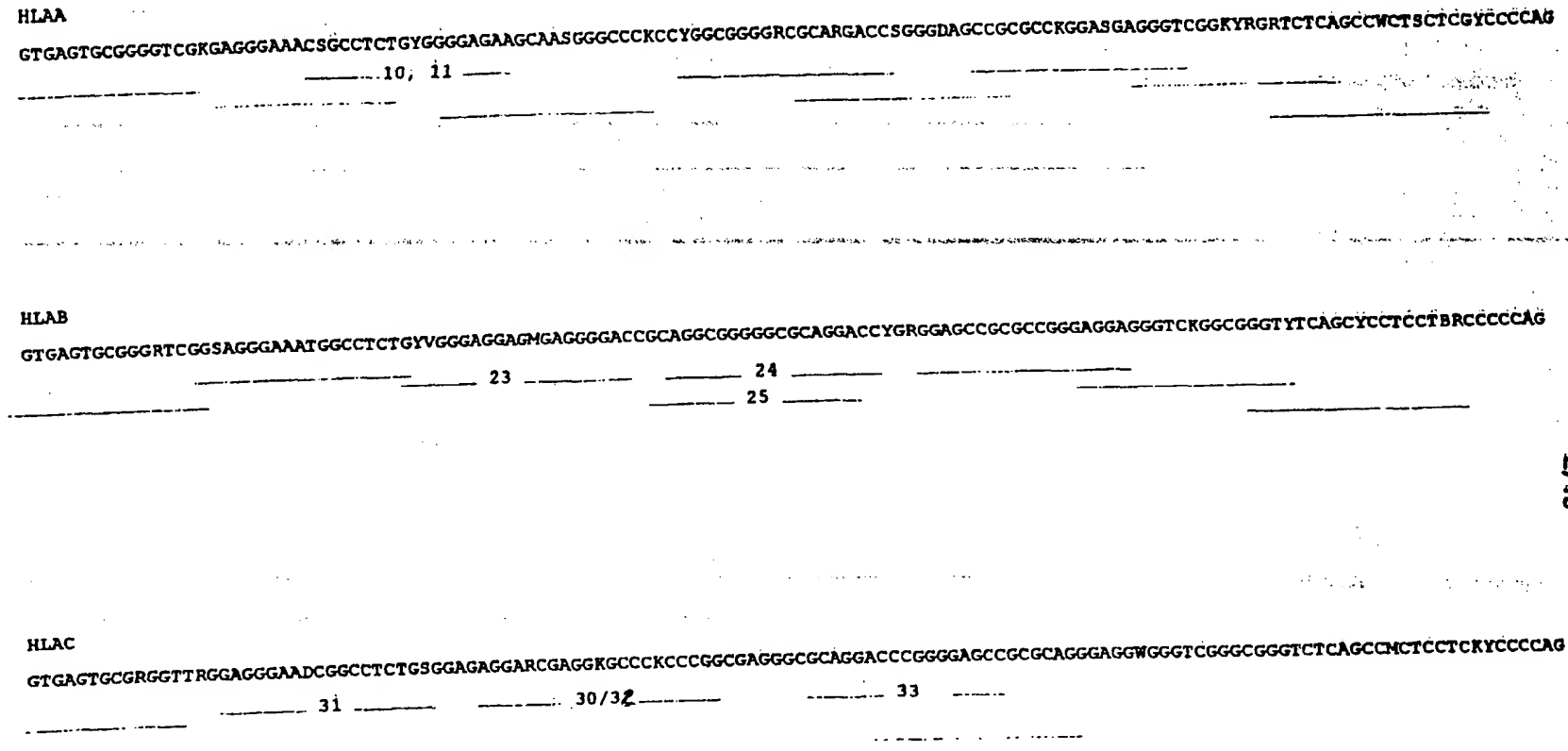


Fig. 2

FIGURE 3
CONSENSUS SEQUENCES OF INTRON 2 WITH AMPLIFICATION PRIMER LOCATIONS

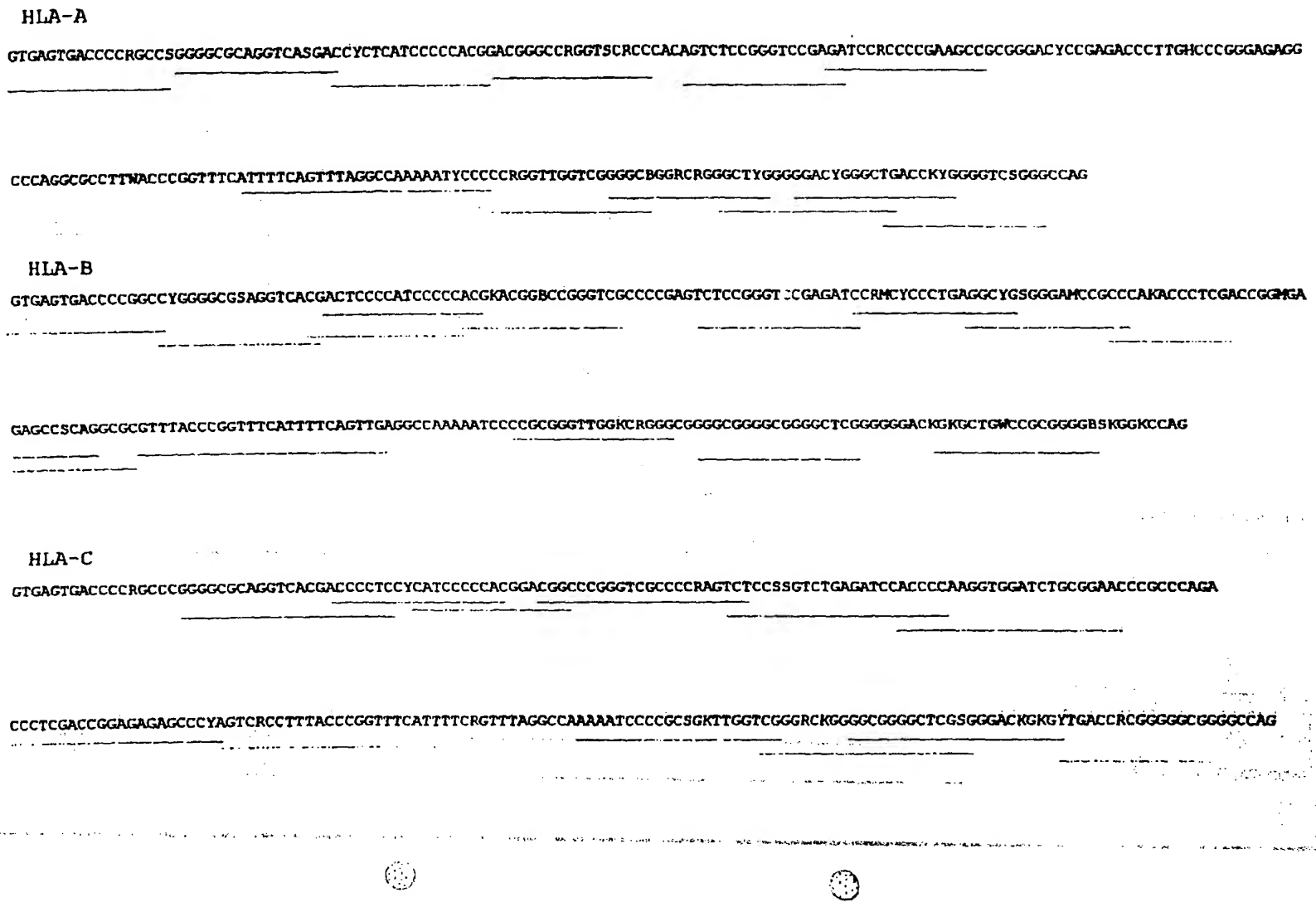


FIG. 4
CONSENSUS SEQUENCE OF INTRON 3 WITH LOCUS-SPECIFIC AMPLIFICATION PRIMER LOCATIONS

Fig. 5A
HLA INTRON 1 Sequence

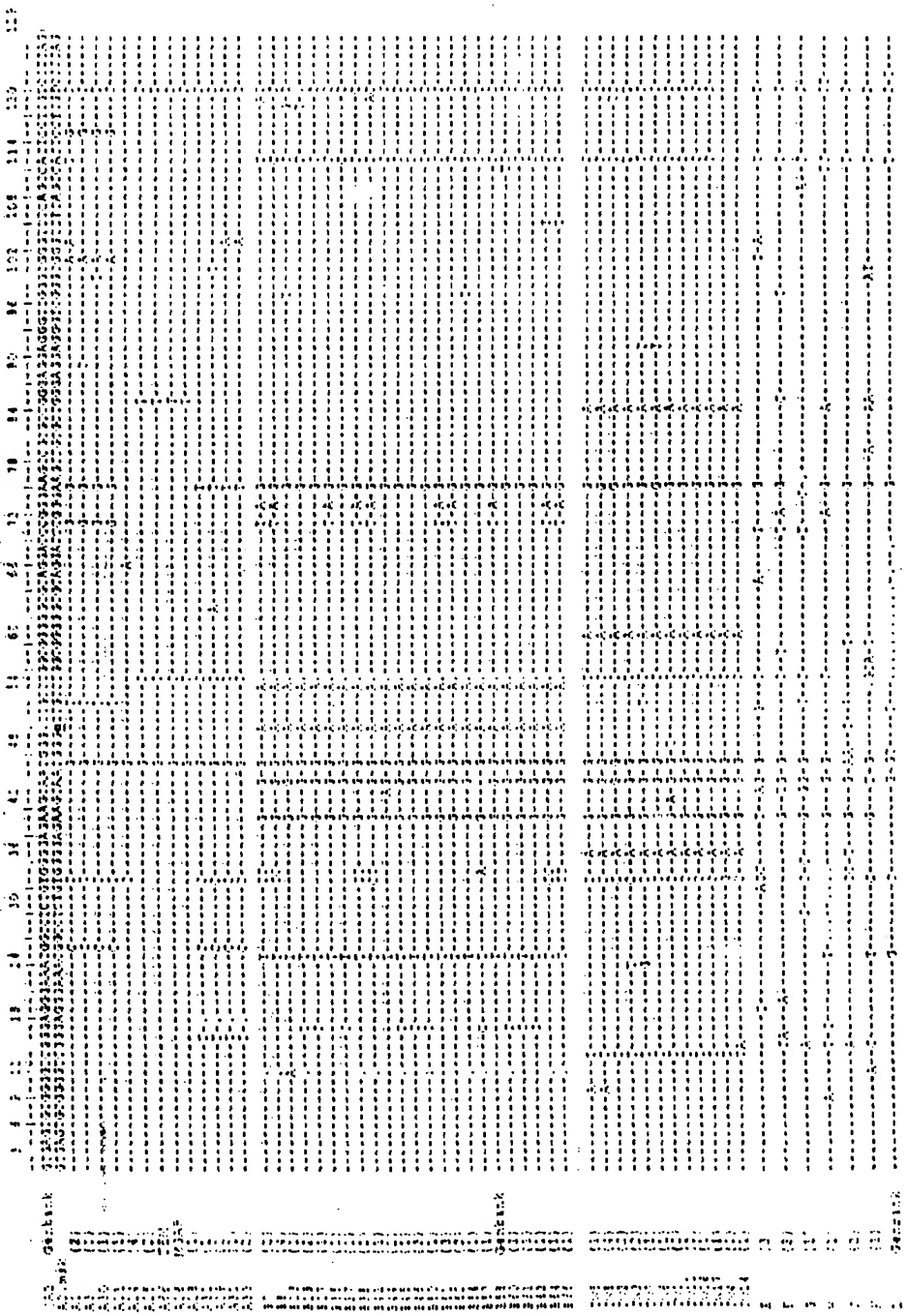


Fig. 5B

HLA Intron 2 (Part I)

Fig. 5C
HLA Intron 2 (Part II)

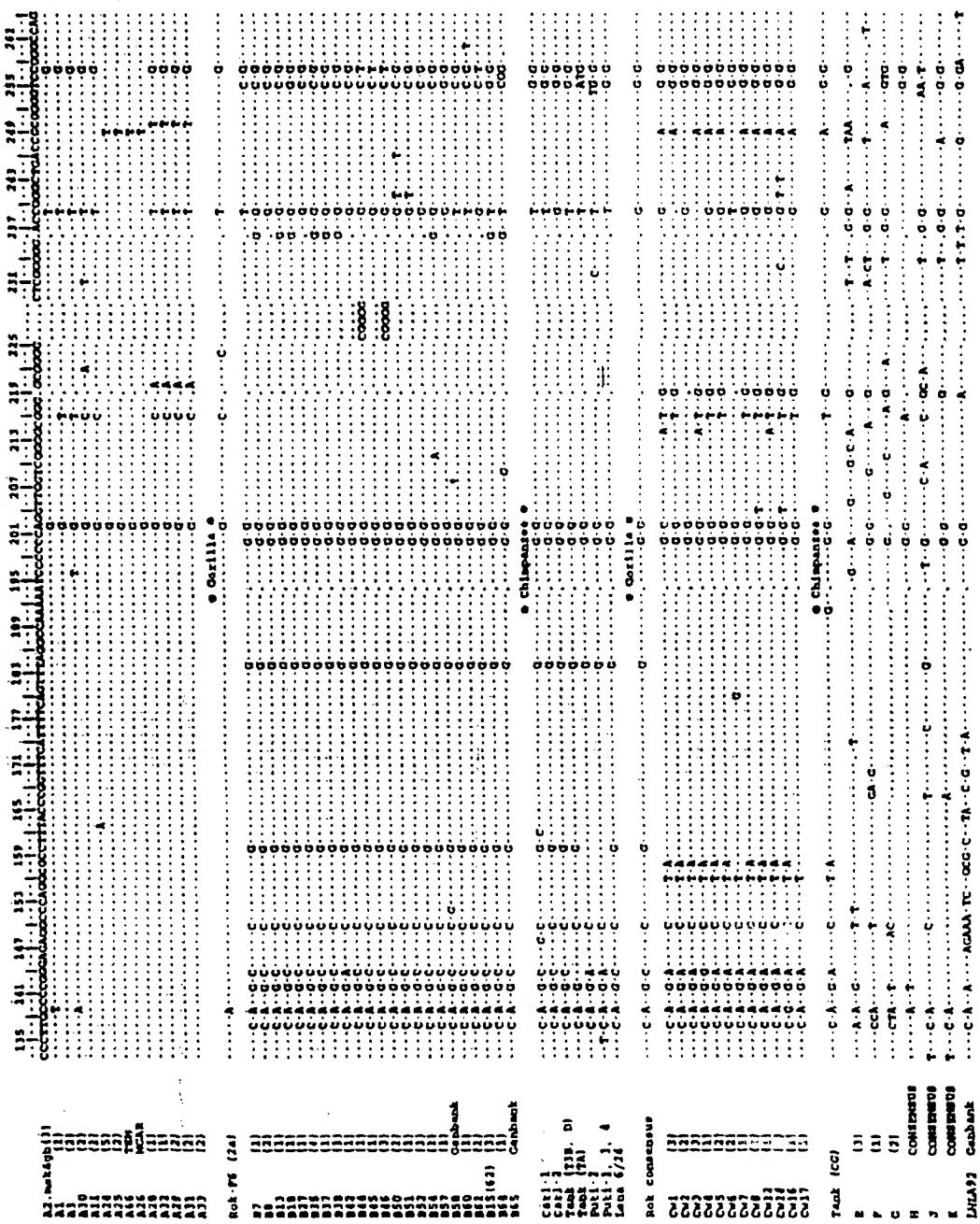


Fig. 5D
HLA Intron 3 (Part I)

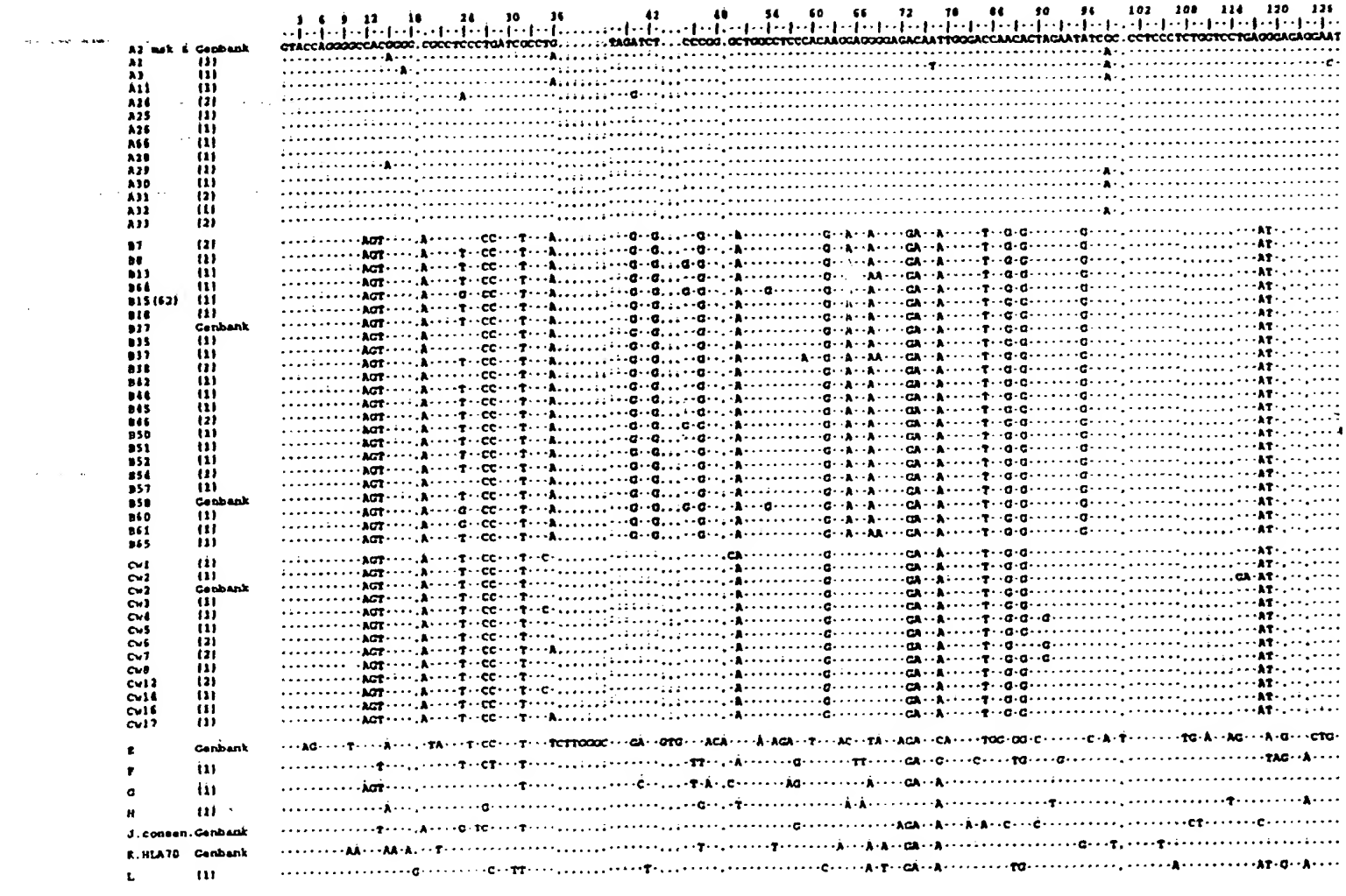


Fig. 5E
HLA Intron 3 (Part II)

Fig. 5F
HLA Intron 3 (Part III)

Fig. 5G
HLA Intron 3 (Part IV)

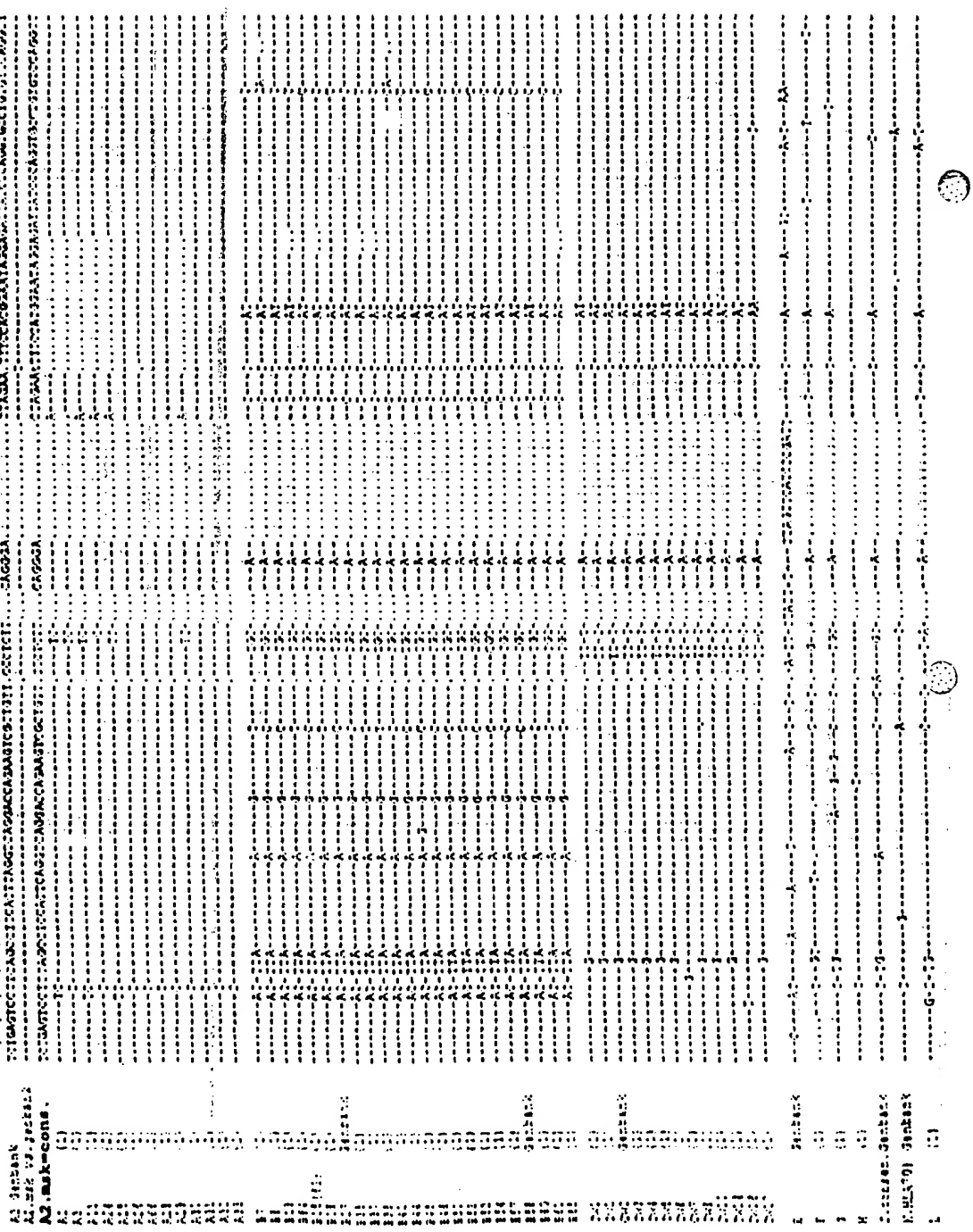
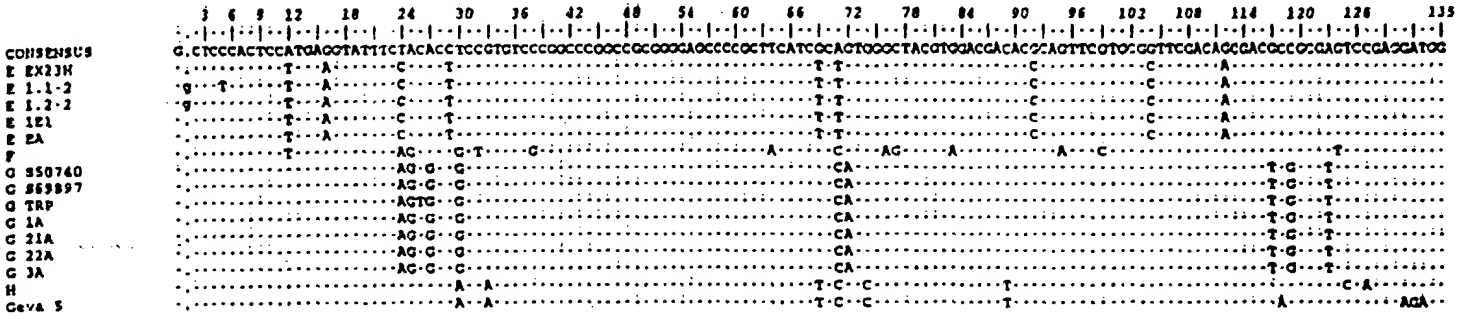


Fig. 5H
HLA Intron 3 (Part V)

Fig. 6

HLA Class I Non Classicals DNA Sequence

Exon 2 (Part I)



Exon 2 (Part II)

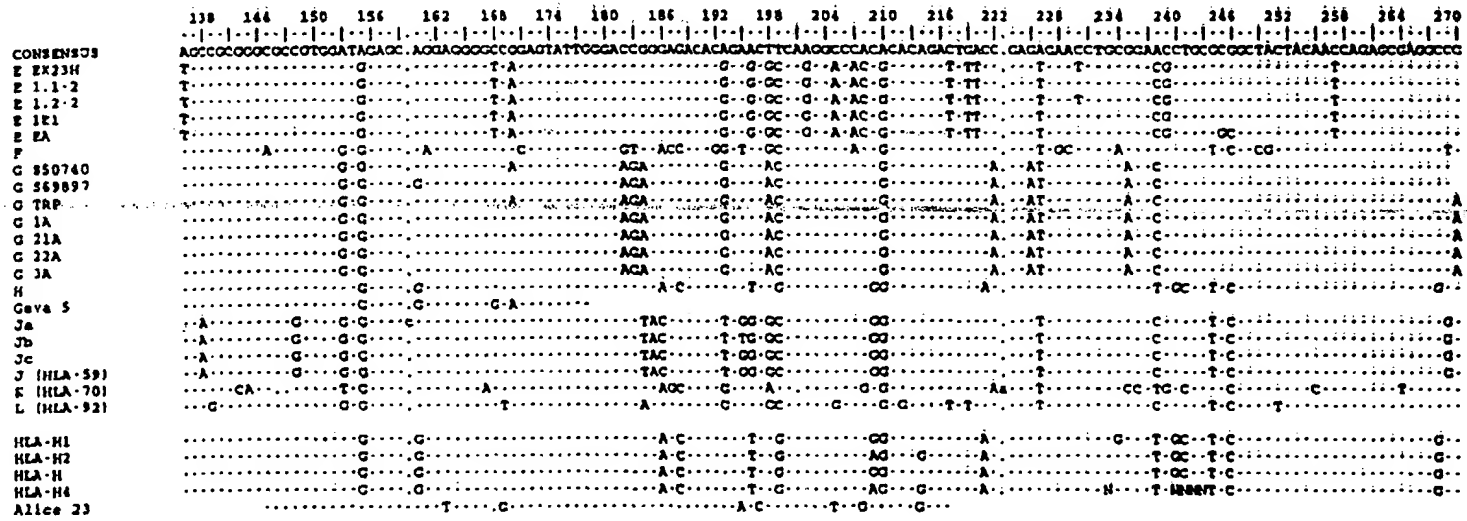
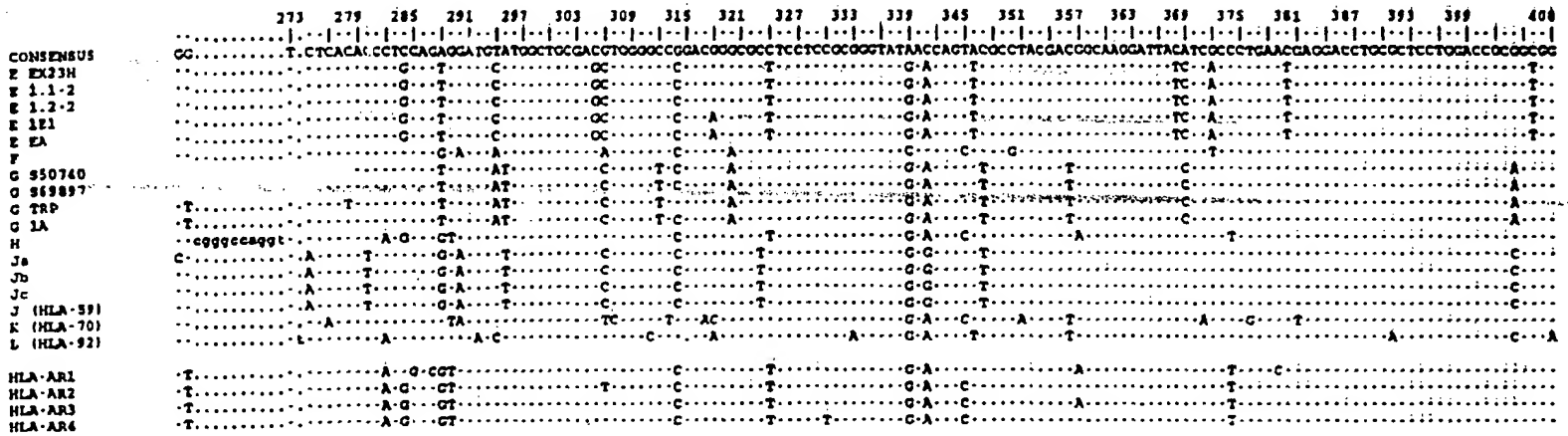


Fig. 7

HLA Class I Non Classicals DNA Sequence

Exon 3 (Part I)



Exon 3 (Part II)

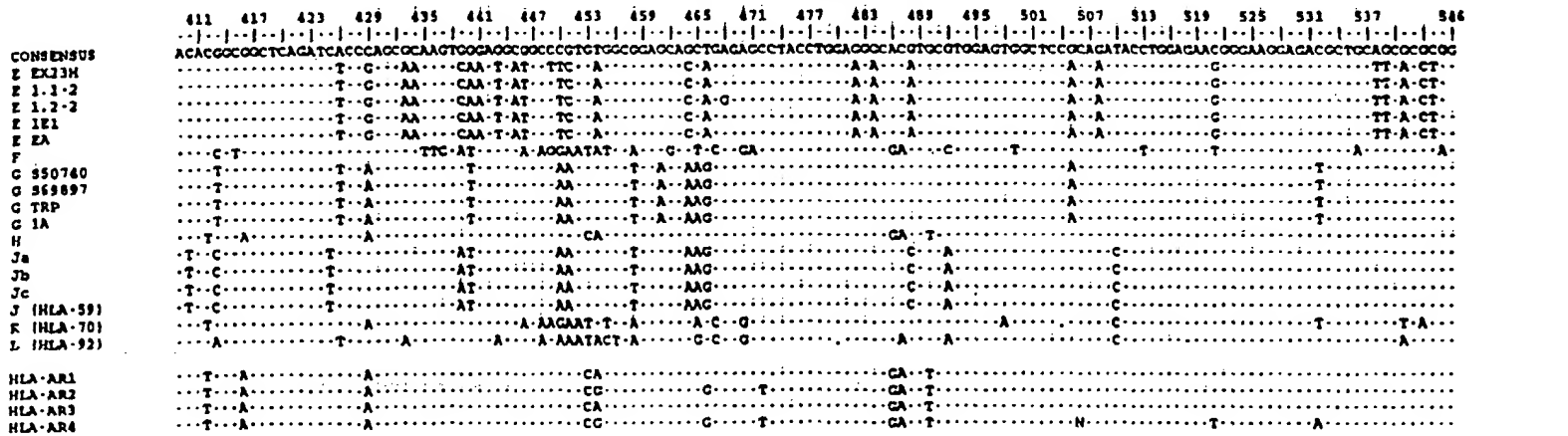


Fig. 8

B-LCL Panel

Dot Number	Name	HLA-A	HLA-B	HLA-C
1	EA	A24	B7	Cw07
2	MZ070782	A24	B14	Cw02,Cw05
3	KAS116	A24	B51	Cw12
4	JESTHOM	A2	B27	Cw01
5	HCHC	A3	B27	Cw01
6	WT100BIS	A11	B35	Cw04
7	DEM	A2	B57	Cw06
8	ZO208915	A25	B18	Cw12
9	KASO11	A1	B37	Cw06
10	ANA1	A28	B53	Cw04
11	E4181324	A1	B52	Cw12
12	WIR076	A2	B57	Cw07
13	SCHU	A3	B7	Cw07
14	HOAR	A26	B8	Cw07
15	WT24	A2	B27	Cw02
16	RHL	A2	B52	Cw15
17	WT8	A3	B7	Cw07
18	LCO81785	A3,A24	B18	Cw05
19	DUCAF	A30	B18	Cw05
20	QBL	A26	B18	Cw05
21	BSH	A68,A30	B42	Cw17
22	COX	A1	B8	Cw07
23	VAVY	A1	B8	Cw07
24	KT17	A2,A11	B35,B62	Cw04,Cw03
25	DEU	A31	B35	Cw04
26	YAR	A26	B38	Cw12
27	FF97387	A29	B44	Cw16
28	PE117	A24	B60,B61	Cw03
29	WT51	A23	B65	Cw08
30	JHAP	A31	B51v	Cw15
31	BOLETH	A2	B62	Cw03
32	BSM	A2	B62	Cw03
33	BM14	A3	B7	Cw07
34	SAVC	A3	B7	Cw07
35	JBUSH	A32	B38	Cw12
36	SPO010	A2	B44	Cw05
37	SWEIG007	A29	B61	Cw02
38	BM16	A2	B18	Cw07
39	JVM	A2	B18	Cw05
40	BM15	A1	B49	Cw07
41	JO528239	A1	B35	Cw04
42	TISI	A24	B35	Cw04
43	BM21	A1	B41	Cw17
44	BKIP	A24	B51,B15	Cw00
45	TUBO	A3,A3	B51	Cw07,Cw15
46	BH	A2	B13	Cw06
47	PLH	A3	B47	Cw06
48	LBUF	A30	B13	Cw06
49	IBM9	A33	B65	Cw08
50	MOU	A29	B44	Cw16
51	PITOUT	A29	B44	Cw16
52	DBB	A2	B57	Cw06
53	HOR	A33	B44	Cw14
54	EK	A2	B44	Cw05
55	HO301	A3	B14	Cw08
56	KOSK	A2	B35	Cw12
57	TEM	A26	B38	Cw12
58	CBM	A2	B45	Cw16
59	SLE005	A2	B60	Cw03
60	CB6B	A1	B62	Cw03
61	31227ABO	A2	B18	Cw07
62	MOV	A2	B38	Cw12
63	WT47	A32	B44	Cw05
64	AMALA	A2	B62	Cw03
65	HHXB	A3	B7	Cw07
66	TABO89	A2	B46	Cw01
67	BTB	A2	B27	Cw01
68	BM9	A2	B35	Cw04
69	MADURA	A2	B60	Cw03
70	LUY	A2	B51	Cw14,Cw00
71	OLGA	A31	B62	Cw01
72	SPACH	A31	B62	Cw01
73	KT12	A24,A31	B51,B35	Cw04,Cw00
74	HID	A2	B60,B61	Cw03,Cw08
75	DKB	A24	B60	Cw03
76	T7526	A2	B46	Cw01
77	T7527	A2	B46	Cw01
78	PMO075	A3,A33	B65	Cw08
79	LMMOS	A33	B14	Cw08
80	EHM	A3	B35	Cw04
81	EA	A3	B7	Cw07
82	HO104	A3	B7	Cw07
83	LEEB	A3	B7	Cw07
84	CALOGERO	A2	B61	Cw02
85	EJJZB	A30	B18	Cw05
86	LO541265	A1	B8	Cw07
87	STEINLIN	A1	B8	Cw07
88	PFO4015	A1	B8	Cw07
89	BOB	A24	B51	Cw15
90	AMELLS	A2	B44	Cw05
91	MLF	A2	B62	Cw03
92	BM92	A25	B51	Cw01
93	BER	A2	B13	Cw06
94	CF996	A2,A3	B14	Cw08
95	WIN	A1	B57	Cw06
96	LBF	A30	B13	Cw06
97	EM7	A2,A3	B60	Cw03
98	MT14B	A31	B60	Cw03
99	LZL	A2	B62	Cw03
100	OLL	A31	B62	Cw01
101	SPL	A31	B62	Cw01
102	ARBO	A3	B57	Cw06
103	KT14	A24,A26	B51;B61	Cw08,Cw14
105	FPAF	A1	B35	Cw04,Cw00
106	MANIKA	A3	B50	Cw06
107	LKT3	A24	B54	Cw01

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/00362

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12Q 1/68; C12P 19/34; C07H 21/04
US CL : 435/6, 91.2; 536/24.33
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 91.2; 536/24.33

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, BIOSIS, CAPLUS, EMBASE
search terms: HLA, typing, intron, amplification, PCR

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BLASCZYK et al. Complete subtyping of the HLA-A locus by sequence-specific amplification followed by direct sequencing or single-strand conformation polymorphism analysis. Tissue Antigens. April 1995, vol 46, pages 86-95. See pages 86-90.	1-16
X ---, P Y	CEREB et al. Locus-specific amplification of HLA class I genes from genomic DNA: locus-specific sequences in the first and third introns of HLA-A, -B, and -C alleles. Tissue Antigens. December 1995, vol. 45, pages 1-11. See entire document.	1-3, 10, 14, 15, 17, 18, 22, 32, 33, 37, 38 --- 1-29, 32-40

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 09 MAY 1996	Date of mailing of the international search report 24 MAY 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer AMY ATZEL Telephone No. (703) 308-0208

Form PCT/ISA/210 (second sheet)(July 1992)*

BNSDOCID: <WO___972364SA1_I_>

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/00362

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y, A	GERAGHTY et al. The HLA class I gene family includes at least six genes and twelve pseudogenes and gene fragments. J. Immunol. September 1992, vol. 149, pages 1934-46. Entire document.	1-29, 32-40
Y	LAWLOR et al. Ancient HLA genes from 7,500-year-old archaeological remains. Nature. February 1991, vol. 349, pages 785-788. See pages 785-787.	1-29, 32-40
Y, A	MALISSEN et al. Exon/intron organization and complete nucleotide sequence of an HLA gene. Proc. Natl. Acad. Sci. USA. February 1982, vol. 79, pages 893-97. Entire document.	1-29, 32-40
Y	JOSEPH et al. Classification of Mutations at the HLA-A locus by use of the polymerase chain reaction. Environmental and Molecular Mutagenesis. July 1993, vol. 22, pages 152-56. See page 153.	1-29, 32-40
Y	SUMMERS et al. HLA Class I Noncoding Nucleotide Sequences, 1992. Eur. J. Immunogenetics. Blackwell Scientific Publications. June 1993, vol. 20, pages 201-240. See pages 211-221.	1-29, 32-40

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/00362

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 30 and 31
because they relate to subject matter not required to be searched by this Authority, namely:

Claims describe possible mental steps involved in selection of primers for amplification.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.